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DISEASES OF CHILDREN

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Incontinentia Pigmenti (Bloch-Sulzberger Syndrome)
Robert S. Mendelsohn, Roland S. Medansky, and M. Paul Lazar

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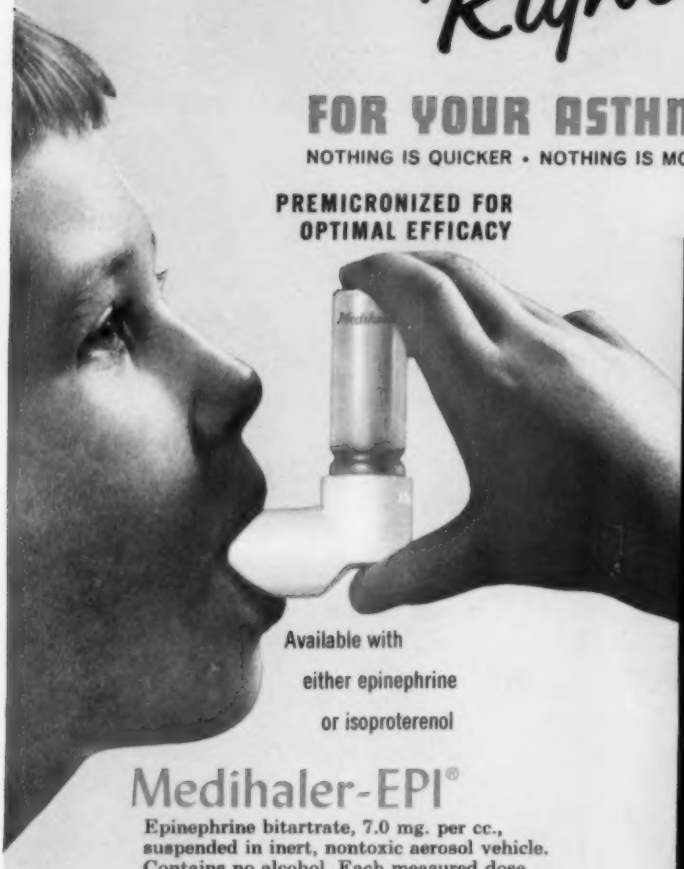

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TABLE OF CONTENTS

VOLUME 97 MARCH 1959 NUMBER 3

ORIGINAL ARTICLES

PAGE

Isolation of Enteropathogenic Escherichia Coli from Mothers and Newborn Infants
Merlin L. Cooper, M.D.; Helen M. Keller, M.S.; Edward W. Walters, M.S.; John C. Partin, B.S., and Donald E. Boye, Cincinnati, with the Assistance of Evelyn W. Perry, A.B.; Jacqueline J. Partin, B.S.; George Nagao, A.B., and Charles Neilson, B.S. 255

Fetomaternal Transfusion
Howard A. Pearson, M.D., and Louis K. Diamond, M.D., Boston 267

Attempted Abortion with Aminopterin (4-Amino-Pteroylglutamic Acid)
Josef Warkany, M.D.; Pierre H. Beaudry, M.D., and Stephen Hornstein, M.D., Cincinnati 274

Calcification of Intervertebral Disks in Children
A. Asadi, Ph.D., M.D., Ann Arbor, Mich. 282

Subclinical Pneumocystis Pneumonitis
Walter H. Sheldon, M.D., Atlanta 287

The Association of Various Factors and Hypernatremic Diarrheal Dehydration
Martha N. Franz, M.D., and William E. Segar, M.D., Indianapolis ... 298

Lysozyme Levels in Cystic Fibrosis of the Pancreas
Morris N. Green, Ph.D.; Lucas L. Kulczycki, M.D.; Martin Nemer, Ph.D.; Sheila Blume, M.D., and Harry Shwachman, M.D., Boston, with the Technical Assistance of Howard Kremin and Barbara Williams 303

Vasopressin-Resistant Diabetes Insipidus
S. A. Kaplan, M.D.; A. M. Yuceoglu, M.D., and J. Strauss, M.D., Brooklyn 308

Hypnosis as an Adjunct to Anesthesia in Children
Milton J. Marmer, M.D., Beverly Hills, Calif. 314

Bacterial Flora of the Nasopharynx in Children
Cecilia Y. Willard, Ph.D., and Arild E. Hansen, M.D., Ph.D., Galveston, Texas 318

The Nonspecific Inflammatory Cycle in the Neonatal Infant
D. V. Eitzman, M.D., and Richard T. Smith, M.D., Gainesville, Fla. 326

Skin-Test Prediction as a Teaching Aid
Morton Zall, M.D., and Ernest M. Heimlich, M.D., Los Angeles 335

CASE REPORTS

Atropine Poisoning with Psychosis
Maria Emma Gamboa, M.D., and Edward W. Gamble III, M.D., Birmingham, Ala. 342

Phenylketonuria Treated from Earliest Infancy
Frederick A. Horner, M.D., and Charles W. Streamer, M.D., Denver . 345

Incontinentia Pigmenti (Bloch-Sulzberger Syndrome)
Robert S. Mendelsohn, M.D.; Roland S. Medansky, M.D., and M. Paul Lazar, M.D., Chicago 348

REGULAR DEPARTMENTS

Abstracts from Current Literature 353
News and Comment 373, 374

A. M. A.
Journal of Diseases of Children

VOLUME 97 MARCH 1959 NUMBER 3

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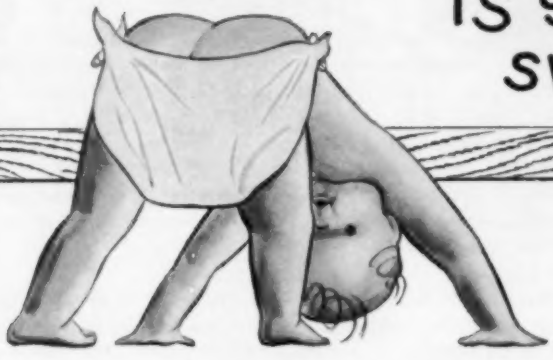

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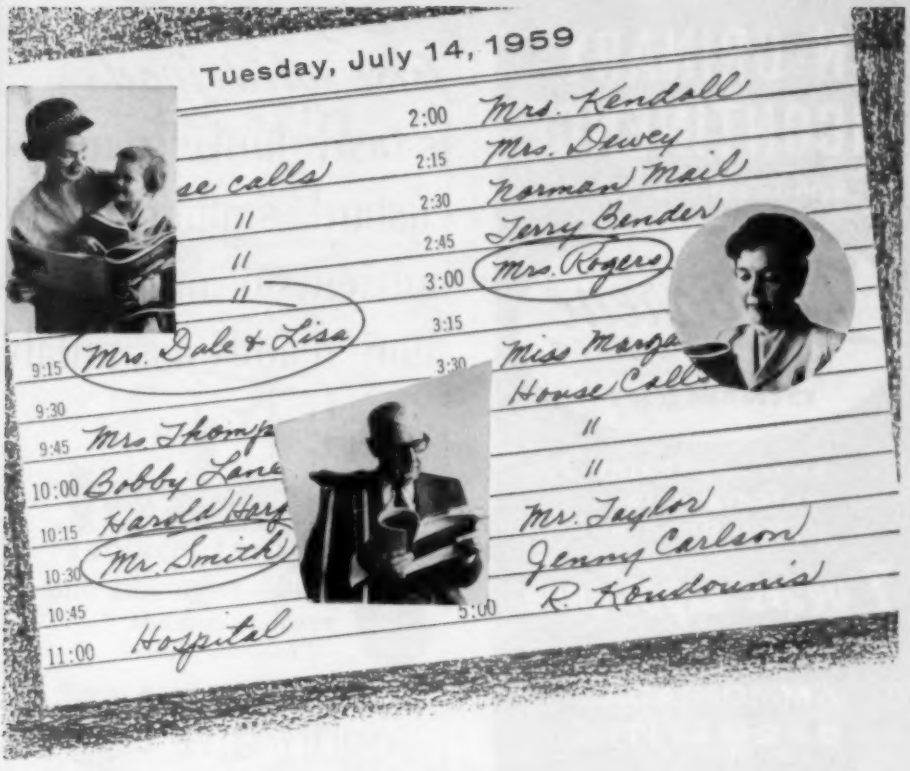

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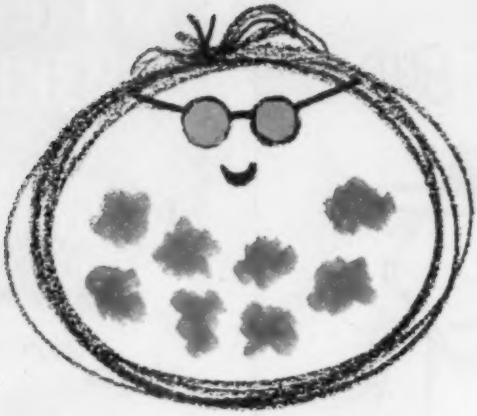

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Isolation of Enteropathogenic Escherichia Coli from Mothers and Newborn Infants

MERLIN L. COOPER, M.D.; HELEN M. KELLER, M.S.; EDWARD W. WALTERS, M.S.; JOHN C. PARTIN, B.S., and DONALD E. BOYE, Cincinnati

With the Assistance of Evelyn W. Perry, A.B.; Jacqueline J. Partin, B.S.; George Nagao, A.B., and Charles Neilson, B.S.

Studies by various investigators of infants from whom enteropathogenic Escherichia coli (EPEC) were isolated reveal that many infants were in the first weeks of life and that the majority had an associated diarrhea. Cathie and MacFarlane [1] (1951) studied 264 cases of infantile diarrhea and found E. coli O111:B_4 in stool cultures of 16.7% in each of the first two months of life. Shanks and Studzinski [2] (1952) studied 158 infants with diarrhea, culturing stools for E. coli O55:B_5 and O111:B_4, and found these serotypes in 25.0% of infants under 4 weeks of age. Thomson, Watkins, and Gray [3] (1956) searched for E. coli O26:B_6, O55:B_5, and O111:B_4 in a group of infants, 77.0% of whom were in the first three months of life, and they found 20 infants (18.0%) harboring one of these three types. In 8 of these 20 infants there was an associated diarrhea. Curtin and Clifford [4] (1956), in a study to determine the incidence of E. coli O26:B_6, O55:B_5, O111:B_4, and O127:B_8 among neonatal patients, detected 2 of 91 infants in the first week of life excreting Types O26:B_6 and O55:B_5, respectively. These two infants did not have diarrhea at the time stool was obtained for culture. Cooper, Keller, and Walters [5] (1957) studied 169 infant patients, the majority with diarrhea, from whom one of nine serotypes of EPEC were isolated, and found that 43.8% were in their first three months of life. A recent review and classification of these infants according to their ages by weeks reveals that in the first, second, third, and fourth weeks of age, there were four, six, six, and five infants, respectively, all but one with diarrhea. In the second, third, fourth, fifth, sixth, and seventh months of life there were 23, 27, 18, 16, 7, and 5 infants, respectively, and all but 6 had diarrhea.

Since the source of infection of young infants is usually unknown, except in institutional epidemics, and since a significant number of cases of diarrhea associated with the presence of EPEC occur early in life when the infants have limited opportunity for exposure, the question arises regarding the source of infection in their immediate environment. We wondered whether mothers, who are usually in closest contact with the infants, might be the sources of infection, perhaps as asymptomatic intestinal carriers. Our previous experience, as well as that of others, has shown that an occasional adult harbors EPEC in the intestinal tract without evidence of infection, especially in epidemics of diarrhea in infants,

Submitted for publication July 29, 1958.

The Children's Hospital Research Foundation and the Department of Pediatrics, University of Cincinnati College of Medicine.

where personnel in contact with the patients are detected as asymptomatic carriers of the same serotype. Studies by McNaught and Stevenson [6] (1953) of adults with diarrhea in which they cultured for six serotypes of EPEC showed three types as the apparent etiologic agent in 15 adults. In our study [7] (1955) of a hospital epidemic in which E. coli O127:B_8 was detected as the etiologic agent, 82 persons on the involved infants' ward were cultured and one nurse was found to be an asymptomatic intestinal carrier.

A review of the literature reveals that EPEC have been isolated from stool cultures of a few mothers of young infants. Taylor, Powell, and Wright [8] (1949) report the detection of E. coli O111:B_4 in cultures of stools from three mothers, two of whom had young infants with diarrhea, from whose stool cultures the same serotype was isolated. Stevenson [9] (1952) reports the detection of E. coli O111:B_4 in stool cultures of 4 among 44 apparently healthy mothers, whose infants, aged 6 days, 9 days, 3 months, and 6 months, had diarrhea and stool cultures positive for E. coli O111:B_4. Thomson, Watkins, and Gray [3] (1956) studied 17 family contacts of 20 young infants from whom EPEC were isolated. Eight of the twenty infants had diarrhea, and 5 of the 17 family contacts, including 3 mothers, were found to be excreting the same serotypes as were the infants. These data indicate that EPEC may not only act as the etiologic agent in an occasional adult with diarrhea but may also parasitize the intestinal tract of an occasional adult who is in contact with infants with acute diarrhea. Since our review of the literature did not reveal information regarding the possible role of mothers as sources of infection for their newborn infants at the time of birth, we planned a study during the summer of 1957 to determine whether a group of mothers harbored EPEC at the time of the birth of their infants and whether the newborn infants acquired the same serotypes during their first four days of life.

While we were compiling the data accumulated in this study, our attention was called to a report by Ocklitz and Schmidt [10] (1955), in Germany, in which cultures of the "last stool before birth" revealed that 7.0% of mothers were harboring EPEC and cultures of the "first stool after birth" revealed that 14.0% of mothers were harboring these micro-organisms. A more recent publication by Ocklitz and Schmidt [11] (1957), which appeared after our study was completed, contains preliminary results from the bacteriological study of stools from 111 mothers and their newborn babies. The specimens were obtained from "women coming for consultation to the antenatal clinics" and from the newborn babies and were cultured for E. coli O25:L_{11}, O26:B_6, O55:B_5, and O111:B_4. In five cases EPEC were found in the stools of mothers alone and in three cases, in the infant only. "The coincidence of positive results in mother and baby was less often seen." In one case the same E. coli serotype was found in the mother's stool on the last day before her admission to the clinic and, after delivery, in the rectal swab of the newborn infant.

With the cooperation of Dr. Stanley T. Garber, of the Obstetrical Service, and Dr. James M. Sutherland, of the Newborn Service, of the Cincinnati General Hospital, mothers were studied during June, July, and August, 1957, by obtaining vaginal and rectal swabs shortly before and shortly after delivery and two additional rectal swabs during the three days following delivery. The newborn infants of these mothers were studied by obtaining three nasopharyngeal and three rectal swabs during the first four days of life. All the swabs were cultured and studied for the presence of 11 serotypes of EPEC reported by Edwards and Ewing [12] (1955) and by Ewing, Tatum, and Davis [13] (1957) in association with epidemic and sporadic cases of diarrhea among infants. Follow-up studies, with the cooperation of Dr. Carl S. Wilzbach, Cincinnati Health Commissioner, and Miss Catherine E. Ludlow, Director of Nurses of the Cincinnati Health Depart-

ment, entailed weekly visits by nurses to the homes of "positive" * families during the first three months in the lives of the infants, at which times rectal swabs were obtained from mothers and infants for culture, together with information regarding diarrhea. Follow-up studies of "negative" † families entailed obtaining rectal swabs from mothers and infants when the infants visited pediatric clinics at 6 weeks of age for pediatric evaluation or at any other time that diarrhea might occur during their first three months of life.

Methods

Rectal and vaginal specimens were obtained on swabs which were placed immediately in test tubes containing 2.0 ml. of sterile buffered glycerin preservative solution.[14] If more swabs were obtained during one day than could be cultured that day they were stored temporarily at —20 C. Rectal swabs were cultured on 2 Petri dishes each of Sorbitol Agar (Difco) and MacConkey Agar (Difco) and 1 Petri dish of blood agar prepared with Trypticase Soy Agar (Baltimore Biological Laboratory) plus 5.0% fresh defibrinated rabbit blood, after which the swab was placed in Tetrathionate Broth Base (Difco). After incubation the latter was subcultured onto one Petri dish containing Brilliant Green Agar (Difco) to detect salmonellae. However, since salmonellae were not detected during the first two months of this study, further search for these micro-organisms was discontinued. Vaginal swabs were cultured in the same way as were the rectal swabs, except that Tetrathionate Broth and Brilliant Green Agar were not used. Additional detailed methods are described in our previous publications[5,7] (1957, 1955).

All rectal swabs from personnel and from persons with diarrhea or a recent history of diarrhea were cultured for salmonellae and shigellae in addition to EPEC, as outlined in an earlier publication[8] (1957).

The methods for preparation and use of the nasopharyngeal swabs are found in a publication by two of us concerning severe staphylococcal infections in young children[15] (1958). In the present study rabbit blood was not added to Trypticase Soy Broth (Baltimore Biological Laboratory), since we were primarily interested in detecting E. coli, which do not require blood for growth. After incubation overnight this culture was studied in the same way as were cultures of rectal swabs, except that the swab was not placed in Tetrathionate Broth.

Isolation and Serologic Identification of Enteropathogenic E. Coli.—If possible, at lease six colonies with coliform characteristics or with different colonial characteristics were picked from each of five Petri-dish cultures and tested directly by macroscopic slide agglutination with polyvalent E. coli antiserum prepared in our laboratory. This antiserum was prepared in the same way as described previously,[6] except that 11 rather than 9 serotypes of EPEC were used. These 11 serotypes were E. coli O26:B_6, O55:B_5, O86:B_7, O111:B_4, O112a,O112b:B_{12}, O112a,O112c:B_{11}, O119:B_{14}, O125:B_{15}, O126:B_{16}, O127:B_8, and O128:B_{12}. If agglutination occurred with the polyvalent antiserum, the same colony was picked to a Sorbitol Iron Agar (Difco) slant and a Triple Sugar Iron Agar (Baltimore Biological Laboratory) slant, with both surface and stab inoculations. After incubation overnight the cultures in the Sorbitol Iron Agar which did not give evidence of the production of hydrogen sulfide and which did show the typical coliform reactions in the matching Triple Sugar Iron Agar culture were tested for macroscopic slide agglutination with our polyvalent E. coli antiserum. If prompt agglutination occurred additional serologic studies, similar to those reported by us previously,[5] were made to determine whether the culture was one of the 11 serotypes used in preparation of the polyvalent antiserum.

Results

Detection of Enteropathogenic E. Coli Among Mothers and Infants in Hospital.

Between June 8 and Sept. 1, 1957, vaginal and rectal swabs were obtained from 361 mothers while in the Cincinnati General Hospital for the birth of their infants and nasopharyngeal and rectal swabs were ob-

TABLE 1.—*Detection of Enteropathogenic E. Coli Among Mothers and Their Infants*

	No. Studied	Enteropathogenic E. Coli					
		Mothers and/or Infants					Totals
Mothers	361	18	27	0	1	--	46 (12.7%)
Infants	355	18 *	0	3	--	2	23 (6.5%)
Totals	716	36	27	3	1	2	69 (9.6%)

* Same serotypes as detected in mothers.

* "Positive" family indicates a case in which enteropathogenic E. coli was detected in rectal swab cultures of either mother or infant or both at the time of birth of the infant.

† "Negative" family indicates a case in which enteropathogenic E. coli was detected in rectal swab cultures of mother or infant at the time of the birth of the infant.

tained from 355 of these newborn infants (Table 1). EPEC were isolated from 46 (12.7%) of the mothers and 23 (6.5%) of the infants. Eighteen of the positive infants harbored the same serotypes as did their mothers.

Initial Detection of Enteropathogenic E. Coli in Mothers in Hospital.—Cultures of rectal swabs obtained just before and just after delivery and on the first and second day after delivery facilitated the initial detection of EPEC in 56.5%, 73.9%, and 91.3% (Table 2) of the mothers found to

TABLE 2.—*Time of Initial Detection of Enteropathogenic E. Coli in Cultures from Forty-Six Mothers*

1st Positive Culture	Vaginal Swabs	Rectal Swabs		
At delivery				
Before	2	16 (34.8%)	(56.5%)	
After	1	10 (21.7%)		(73.9%)
After delivery				
1 Day		8 (17.4%)		(91.3%)
2 Days		8 (17.4%)		
3 Days		3 (6.5%)		
4 Days		1 (2.2%)		
Total no. mothers	3	46		

be harboring the micro-organisms in their intestinal tracts. Rectal swabs on the third day after delivery resulted in the initial detection of three additional mothers. The same serotypes were isolated from rectal swabs obtained from seven mothers just before and just after delivery. Vaginal swabs obtained from two mothers just before delivery and from one mother just after delivery were positive for EPEC.

The serotypes isolated from the 46 mothers and the frequency of their isolation are shown in Table 3, where it is seen that serotypes associated with sporadic and epidemic cases of diarrhea in infants were isolated from 23 (50.0%) of the 46 mothers. The remainder of the mothers were harboring serotypes known to be associated so far only with sporadic cases of diarrhea. E. coli O125:B_{15} and O112ab:B_{13} were isolated most frequently.

TABLE 3.—*Enteropathogenic E. Coli from Mothers and Their Infants*

Serotypes	Mothers	Infants	Totals
O112ab:B_{12}	10	7	17
O125:B_{15}	11	3	14
O127:B_8*	7	2	9
O126:B_{16}*	5	3	8
O111:B_4*	4	3	7
O55:B_5*	2	2	4
O86:B_7	1	2	3
O128:B_{12}*	2	1	3
O26:B_6*	2	0	2
O112ac:B_{13}*	1	0	1
O119:B_{14}	1	0	1
Totals	46	23	69

* Serotypes associated with epidemic and sporadic cases of diarrhea in infants. The remaining serotypes associated with sporadic cases of diarrhea.

Initial Detection of Enteropathogenic E. Coli in Infants in Hospital.—Cultures of rectal and nasopharyngeal swabs from 355 newborn infants revealed that 23 (6.5%) were harboring EPEC in their intestinal tracts and that 3 of the 23 harbored EPEC in the nasopharynx. These infants acquired EPEC very early in life since 9 (39.1%) of the 23 infants were detected initially by cultures of rectal swabs obtained on the second day of life, 8 (34.8%) on the third day of life, and 5 (21.7%) on the fourth day of life (Table 4).

The serotypes isolated from the 23 infants and the frequency of their isolation are shown in Table 3, where it is seen that 11 (47.8%) harbored serotypes associated with epidemic and sporadic diarrhea in infants; the other 12 harbored serotypes associated with sporadic cases, and serotype O112ab:B_{13} was isolated most frequently.

TABLE 4.—*Time of Initial Detection of Enteropathogenic E. Coli in Cultures from Twenty-Three Newborn Infants*

First Positive Culture	Nasopharyngeal Swabs	Rectal Swabs		
Days after birth				
1st	1	9 (39.1%)	(73.9%)	
2d	1	8 (34.8%)		(95.6%)
3d	1	5 (21.7%)		
4th		1 (4.3%)		
Total no. infants	3	23		

TABLE 5.—*Relation of Initial Isolations of Same Serotype of Enteropathogenic E. Coli from Rectal Swab Cultures of Eighteen Mothers and Their Newborn Infants*

Mothers Initial Isolations	No.	Infants Initial Isolations: Day of Birth	Days After Birth: 1	2	3	7	No.
At delivery							
Before	8	0	3	3	1	1	8
After	3	0	1	2			3
After delivery							
1 day	3	0	2		1		3
2 days	4	0	1	1	2		4
Totals	18	0	7	6	4	1	18

Same Serotypes Isolated from Mothers and Infants.—The time of the initial isolation of the same serotypes from cultures of rectal swabs from 18 mothers and their infants (Table 5) shows that EPEC were detected in 11 mothers shortly before and shortly after delivery and in 9 of their infants on the first and second days after birth. In addition, EPEC were isolated from the remaining seven mothers and from four of their infants on the first and second days after delivery.

Influence of Multiple Rectal Swab Cultures on Detection of Enteropathogenic E. Coli.—Thirty-three (71.7%) of the forty-six mothers and seventeen (73.9%) of the twenty-three infants with rectal swabs positive for EPEC were detected with the first culture while in the hospital at the time of the birth of the infants. Obtaining a second rectal swab facilitated detection of an additional 11 (23.9%) of the positive mothers and 5 (21.7%) of the positive infants. A third rectal swab resulted in the detection of two additional positive mothers (4.3%) and one positive infant (4.3%). Securing more than three rectal swabs on any one mother or infant did not increase the number of positive persons while in the hospital.

Study of Personnel.—Rectal swab cultures from 114 personnel were studied for the presence of shigellae, salmonellae, and EPEC. These persons had contact with mothers or infants in the delivery rooms, nurseries, and wards or worked in these areas, and they included physicians, registered nurses, student nurses, ward helpers, ward clerks, and the medical students and laboratory technicians who assisted in these studies. E. coli 055:B_5 and O111:B_4 were isolated from two student nurses who were then removed from the service. Salmonellae and shigellae were not detected in any of these personnel.

Postdismissal Studies of Mothers "Positive" in Hospital.—During the first three months post partum EPEC were isolated from rectal swabs from 13 of 38 mothers, 11 of whom were still harboring the same serotypes as had been isolated when they were in the hospital for the birth of their babies. Two of these mothers had lost the serotypes detected at the time of the birth of their infants and had acquired a new serotype. Seven mothers harbored the same serotypes as did their infants postdismissal.

Postdismissal Studies of 21 Mothers "Negative" in Hospital.—EPEC were not isolated from these mothers while they were in the hospital for the birth of their infants. E. coli 055:B_5 was isolated from the rectal swab of only one of the newborn infants. During the first three months at home EPEC were isolated from cultures of rectal swabs of four of these mothers: two mothers and infants harbored the same serotypes, a third mother and infant harbored different serotypes, and the fourth mother, harboring O55:B_5, was the mother of the infant who had harbored E. coli O55:B_5 shortly after birth.

Postdismissal Studies of Infants "Positive" in Hospital.—During the first three months in the lives of these infants rectal swabs were obtained from 18 from whom EPEC had been isolated shortly after birth in the hospital. EPEC were isolated from 10 of these infants, 8 of whom were still harboring the same serotypes detected while they were in the hospital. The other two infants had lost the serotypes harbored by them in the hospital, and each had acquired a new serotype. Seven of these infants

harbored the same serotypes as did their mothers postdismissal.

Postdismissal Studies of 63 Infants "Negative" in Hospital.—EPEC had been isolated from 23 of the mothers of these infants (while they were in the hospital for the birth of these infants); however, all rectal swab cultures of their newborn infants had been negative during their first four days of life in the hospital. During the first three months at home EPEC were isolated from cultures of rectal swabs from 18 (26.6%) of these 63 infants. The same serotypes had been acquired by 4 of these 18 infants as had been harbored by their mothers at the time of the birth of these infants, while three additional infants and their mothers harbored the same serotypes postdismissal.

Incidence of Diarrhea in Relation to Simultaneous Positive Rectal Swab Cultures.

In the hospital at the time of the birth of the infants diarrhea occurred in only 1 of 46 mothers and 1 of 23 infants (Table 6) in association with the simultaneous detection of EPEC in cultures of rectal swabs. During the first three months at home, follow-up studies were made on 18 infants who had been "positive" and on 63 infants who had been "negative" during their first four days of life in the hospital. Among the 18 infants previously "positive," diarrhea occurred in 1, and among the 63 infants previously "negative," diarrhea occurred in 29. A review of our studies on the mothers of the 63 infants at the time of hospitalization showed that 23 of these mothers had harbored EPEC at that time. Only one infant of the 23 "positive" mothers developed diarrhea during the first three months at home, and the rectal swab culture was negative at the time of diarrhea, while 28 infants of the 40 "negative" mothers developed diarrhea during their first three months at home, and EPEC were cultured from rectal swabs of 11 of these 28 infants. Salmonellae were not isolated from any of these mothers or infants with diarrhea. Shigella sonnei was isolated from two of the infants with diarrhea, in one infant in conjunction with the isolation of E. coli O55:B_5.

TABLE 6.—*Incidence of Diarrhea in Relation to Simultaneous Detection of Enteropathogenic E. Coli While in the Hospital and During First Three Months at Home*

	In Hospital				Postdismissal	
		Diarrhea			Diarrhea	
Enteropathogenic E. Coli		Present	Positive Culture	Total No. Studied	Present	Positive Culture
Positive						
Mothers	46	1	1	38	1	1
Infants	23	1	1	18	1	0
Negative						
Mothers	315	2	0	21	2	0
Infants	332	6	0	63 (23 *)	1	0
				(40)	28 †	11 ‡
Totals	716	10	2	140	33	12

* Mothers positive in hospital.
† Shigella sonnei isolated from one infant.
‡ E. Coli O55:B_5 and S. sonnei isolated from one infant.

The low incidence of diarrhea and absence of EPEC in infants born of "positive" mothers (2 of 41 infants), the high incidence of diarrhea among infants of "negative" mothers (28 of 40 infants), and the associated presence of EPEC in cultures of rectal swabs of 11 of these 28 infants implies the possibility that "positive" mothers may transmit passively to their infants specific antibodies which may protect some infants during the first few weeks of life.

Study of Additional Infants and Mothers.

An additional 31 infants who were born in the hospital during the first three months of this study and five of their mothers, all of whom were not cultured at that time, were studied after they returned to their homes, because 29 of the infants developed diarrhea. EPEC were isolated from 8 of these 29 infants and Shigella sonnei, from two other infants. Three mothers were harboring the same serotypes as were their infants. Salmonellae were not isolated from any of these infants and mothers.

Study of Siblings.—Rectal swab cultures of 15 siblings of eight infants from whom EPEC had been isolated while in the hospital or subsequently at home revealed that 5 of the 15 were harboring these micro-

organisms, 4 with the same serotype as was isolated from the newborn infant, and 1 with a serotype different from that harbored by the newborn infant but the same as that carried by the mother. Three of the five siblings harboring EPEC had diarrhea.

Study of Mothers of 19 "Positive" Patients in Children's Hospital.—During the period of this study an attempt was made to obtain rectal swabs from mothers of infant patients in the Children's Hospital from whom EPEC were isolated. These micro-organisms were isolated from 5 of 19 mothers, 4 of whom were harboring the same serotypes as were their infants. E. coli O55:B_5 was isolated from three of these four mothers and their infants and O126:B_{16}, from the fourth mother and her infant. E. coli O55:B_5 was isolated most frequently, from 5 of the 19 infants and three of the five mothers. Shigella sonnei was isolated from the grandmother of one infant found to be harboring two serotypes, E. coli O86:B_7 and O125:B_{15}. The grandmother had diarrhea.

Incidence of Multiple and/or Successive Isolations of Serotypes of Enteropathogenic E. Coli.—During the period of this study more than one sterotype was isolated from six mothers and four infants (Table 7). Cultures of rectal swabs from mothers, taken after their return home from the hospital after accouchement, revealed, first, that two mothers (Nos. 6, 205) lost the serotypes they were carrying in the hospital and each acquired a new serotype; second, that two mothers (Nos. 12, 58) retained the serotypes they were carrying in the hospital and each acquired an additional one, and third, that two mothers (Nos. 149, 191) retained the serotypes they harbored in the hospital and each acquired two additional types. Among the infants studied after their discharge home, two (Nos. 205, 287) lost the serotypes they had been carrying in the hospital and each acquired a different serotype. A third infant (No. 33), from whom EPEC was not isolated while in the hospital, acquired two serotypes which were isolated at different times. The fourth infant (No. 191) continued to harbor the same two serotypes at home as had been isolated in the hospital. It would seem that the initial presence of one serotype did not prevent subsequent acquisition of a different type.

Total Number of Persons Studied and Incidence of Detection of Enteropathogenic E. Coli.—During this study, including three months in the hospital and three months postdismissal, a total of 900 persons were studied, including 385 mothers, 386 infants,

TABLE 7.—*Successive and/or Multiple Isolations of Serotypes of Enteropathogenic E. Coli from Mothers and Their Infants During First Three Months*

	In Hospital		Postdismissal			
Patient No.	Mother	Infant	Mother	Days After Delivery	Infant	Days After Birth
6	O125:B_{15}	Negative	O112ab:B_{12}	40	Negative	
12	O127:B_8	Negative	O127:B_8	37	O125:B_{15}	37,43
			O125:B_{15}	43,46		
33	Negative	Negative	Negative		O111:B_4*	50,68
					O128:B_{12}	77
58	O112ab:B_{12}	O112ab:B_{12}	O112ab:B_{12}	24	O112ab:B_{12}	24
			O125:B_{15}	31		
149	O112ab:B_{12}	O112ab:B_{12}	O112ab:B_{12}	16,24	O112ab:B_{12}	16
			O111:B_4 and O86:B_7*	43		
191	O55:B_5	O55:B_5 and O86:B_7	O55:B_5 and O86:B_7	26	O55:B_5 and O86:B_7	12
			O127:B_8	47	O55:B_5	20
205	O127:B_8	O127:B_8	O126:B_{16}	21	O126:B_{16}	21,20
						38
287	O128:B_{12}	O128:B_{12}	Negative		O126:B_{16}	12,37,47

* Diarrhea.

114 personnel, and 15 siblings. EPEC were isolated from cultures of rectal swabs from 15.1% of the mothers, 12.7% of the infants, 1.8% of the personnel, and 33.3% of the siblings. Of the total 900 persons EPEC were isolated from 114 (12.7%). Of the 499 adults cultured, 60 (12.0%) harbored this group. This incidence of the carrier state among adults emphasizes the potential hazard if such persons handle susceptible infants.

Total Specimens Studied.—During the period of this study 2,191 rectal swabs, 689 vaginal swabs, and 958 nasopharyngeal swabs, or a total of 3,838 specimens, were cultured. Many rectal swabs from infants on the day of birth or the day after birth did not give rise to growth on the plating media or the growth did not contain E. coli, and none of these specimens are included in the total of 3,838 specimens. EPEC were isolated from 220 (10.0%) of the 2,191 rectal swabs, 3 (0.4%) of the 689 vaginal swabs, and 4 (0.4%) of the 958 nasopharyngeal swabs.

Summary of Frequency of Detection of Serotypes of Enteropathogenic E. Coli.—Data in Table 8 show the frequency of detection of the 11 serotypes in mothers, infants, personnel, and siblings during the six-month study. There were a total of 126 isolations of EPEC serotypes from a total of 114 persons, E. coli O55:B_5 and O126:B_{16} being found most frequently. Of the 126 serotypes isolated, 62.7% are associated with sporadic and epidemic cases of diarrhea in infants and the remainder have been associated so far only with sporadic cases of diarrhea. Of the 114 persons from whom EPEC were isolated, 74 (64.9%) were harboring types associated with sporadic and epidemic cases of diarrhea.

TABLE 8.—*Summary of Serotypes of Enteropathogenic E. Coli Isolated from 114 Persons, June 8 to Dec. 8, 1957*

E. Coli Serotypes	Mothers	Infants	Personnel	Siblings	Total
O55:B_5*	9	12	1	2	24
O126:B_{16}*	9	9		3	21
O125:B_{15}	13	7			20
O112ab:B_{11}	12	7			19
O111:B_4*	6	8	1		15
O127:B_8*	8	3			11
O86:B_7	3	3			6
O128:B_{12}*	2	2			4
O26:B_6*	2				2
O119:B_{14}	1	1			2
O112ac:B_{11}*	1	1			2
Serotypes	66	53	2	5	126
Persons	58	49	2	5	114

* Serotypes associated with epidemic and sporadic diarrhea in infants. The remaining serotypes associated with sporadic cases of diarrhea.

Efficiency of Polyvalent Enteropathogenic E. Coli Antiserum and Plating Media.—The diagnostic agglutinating polyvalent antiserum prepared in this laboratory and used as described was very satisfactory for the preliminary detection of colonies of the 11 serotypes used in the preparation of this antiserum. Of the colonies picked from Sorbitol Agar, MacConkey's Agar, and blood agar and showing 1+, 2+, 3+, and 4+ macroscopic agglutination with the polyvalent antiserum, it was found that 48.1%, 49.0%, and 44.0%, respectively, of the colonies from these three media showed one of these degrees of agglutination. However, nearly all of the colonies gave 3+ or 4+ agglutination. When the same colonies from these three media were inoculated onto Sorbitol Iron Agar slants and the growth on those slants which did not produce hydrogen sulfide was used for macroscopic agglutination with monospecific antisera, it was found that 89.7%, 90.4%, and 88.3%, respectively, of the cultures from the same three media gave satisfactory rapid agglutination with one of the 11 monospecific antisera. These three media were therefore about equally efficient for the preliminary serologic detection of colonies of enteropathogenic E. coli.

Comment

The data obtained showing that 12.7% of 361 mothers were harboring EPEC in their intestinal tracts at the time of or shortly after the birth of their infants would seem to support the theory that mothers may be the sources of infection for their infants. Additional data showing that

EPEC were isolated from rectal swabs of 6.5% of their newborn infants during the first four days after birth shows that these infants did acquire these micro-organisms very early in life. The data also showed that EPEC were isolated from 40.0% of the newborn infants of "positive" mothers, and in each instance the same serotype was isolated from mother and infant. These findings seem to indicate that in these particular infants their mothers were the immediate sources of their serotypes. This idea is substantiated by the detection of these micro-organisms in the vaginas of three mothers and in the nasopharynges of three newborn infants. Our data would not appear to support the probability of cross infection, since the incidence of initial isolation of EPEC from the infants decreased during the first four days of life.

By studying infants and mothers at home during the first three months in the lives of the infants, an additional 12 infants and their mothers were found to be harboring the same serotypes of EPEC. The detection of a total of 30 instances in which infant and mother harbored the same serotype in their intestinal tracts emphasizes further the family incidence of these micro-organisms. This prevalence of serotypes of EPEC in a large community; the apparent ease with which these micro-organisms are acquired among mothers, their infants, and siblings, and the unexpected incidence of asymptomatic intestinal carriers among mothers increase the potential danger regarding the development of acute intestinal infections among susceptible infants.

In this study, as EPEC were isolated from apparently normal mothers and their newborn infants, a large incidence of diarrhea was anticipated subsequently among these "positive" infants and infants of "positive" mothers. However, the incidence of diarrhea among infants during the first three months in their home environments occurred almost exclusively in infants from whom EPEC had not been isolated during the first four days of life and from whose mothers these micro-organisms likewise had not been isolated in the hospital. In attempting to explain this unexpected finding we have theorized that mothers who are not harboring EPEC in their intestinal tracts may not transmit passively specific antibodies to their infants, who may therefore be susceptible. The reverse possibility is that mothers who are intestinal carriers may transmit passively these antibodies to their offspring and that their infants may therefore be less susceptible. Our data in this respect are too few to permit of any definite conclusion. However, these possibilities are supported by certain reports in the literature. Neter and associates[16] (1955), testing for antibodies to E. coli O111:B_4, O55:B_5, and O26:B_6, found a relationship existing between the presence of E. coli hemagglutinins and the age of the subjects, antibodies being found in over 90.0% of human sera selected at random from persons 12 years of age and older and in less than 14.0% of sera from infants up to the age of 3 months. Placental transfer of these antibodies was demonstrated in only 3 out of 26 cases, and in these instances the maternal antibody titers were from 4 to 16 times higher than those of the cord blood. Yeivin, Salzberger, and Olitzki[17] (1956) found that sera of newborns showed a much lower content of antibodies against E. coli O55:B_5 and O111:B_4 than the corresponding maternal sera. Lindberg and Hunter[18] (1957) found hemagglutinins for at least one of three serotypes of E. coli, O111:B_4, O55:B_5, and O127:B_8, in every specimen of serum from 50 mothers studied, the maximum titer being 1:128. Kauffmann[19] (1954) has advised immunization of pregnant women against enteric pathogens in order to confer passive immunity on newborns.

Further studies would be helpful in attempting to correlate the detection of EPEC in cultures of rectal swabs of mothers and infants, the presence or absence of specific antibodies in sera from both mothers and infants, and the occurrence of diarrhea associated with the simultaneous detection of

EPEC in infants born of postive or negative mothers.

The serotypes of EPEC isolated most frequently from mothers and their infants in the hospital were O112ab:B_{13} and O125:B_{15}, which have been reported so far only in association with sporadic cases of diarrhea. However in the total study, including the follow-up studies at home, E. coli O55:B_5 and O126:B_{16} were detected most frequently, and both types are associated with epidemic and sporadic diarrhea in infants. In our previous studies of young children hospitalized with diarrhea in this community these two serotypes have been isolated most frequently. This difference in the frequency of the serotypes isolated may perhaps reflect the type of persons studied, since in the hospital the mothers and infants were asymptomatic carriers with the exception of one mother and one infant who had diarrhea, while among those studied in their homes the majority of the infants had diarrhea.

At the present time specific antisera afford the only means for the detection and identification of serotypes of EPEC. However certain selective plating media assist in recognizing colonies of E. coli. Although the three plating media used in this study were equally efficient in assisting in the initial detection of colonies of E. coli, we recommend using more than one medium, since each has its advantages and disadvantages. The use of a diagnostic polyvalent antiserum for preliminary detection of colonies of 1 of the 11 serotypes made possible the study of the large number of patients and specimens handled in this investigation. At the present time most bacteriology laboratories may detect serotypes of EPEC in cultures of stools or rectal swabs, since both polyvalent and monospecific antisera are available commercially. The need for examining cultures of stool specimens for this group of micro-organisms, in addition to Salmonella and Shigella, is emphasized by an increasing number of reports of studies in many parts of the world during the past 10 years which show the importance of the association of enteropathogenic E. coli with diarrhea in infants.

Summary

A study was planned for the summer of 1957 to obtain information regarding the possibility of mothers being intestinal carriers of enteropathogenic Escherichia coli (EPEC) and possible sources for the acquisition of these micro-organisms by their newborn infants.

Between June 8 and Sept. 1, 1957, vaginal and rectal swabs were obtained from 361 mothers shortly before and shortly after delivery. Rectal swabs were also obtained during the first four days following delivery.

Nasopharyngeal and rectal swabs were obtained from 355 newborn infants of these mothers during the first four days of life in the hospital.

All specimens were studied for the presence of the 11 serotypes of EPEC associated with epidemic and sporadic diarrhea in infants.

One of the 11 serotypes was isolated from cultures of rectal swabs from each of 46 (12.7%) of the mothers and from cultures of vaginal swabs from three of these mothers. E. coli O125:B_{15} and O112ab:B_{13} were isolated most frequently.

Of these positive mothers, 56.5% were detected when rectal swabs were obtained shortly before and shortly after delivery, 73.9% were detected with an additional rectal swab on the first day after delivery, and 91.3% were detected with another rectal swab on the second day after delivery.

One of the 11 serotypes was isolated from cultures of rectal swabs from 23 (6.5%) of the newborn infants and from the nasopharynges of three of these infants. E. coli O112ab:B_{13} was isolated most frequently.

Of these positive infants, 39.1%, 73.9%, and 95.6%, respectively, were detected initially with cultures of rectal swabs obtained on the first, second, and third days after birth.

During the first four days in the lives of the newborn infants of positive mothers, EPEC were isolated from 40.0% of their

infants, and in each instance the same serotype was isolated from mother and infant.

During a three-month follow-up study at home of 38 mothers and 18 infants from whom EPEC had been isolated at the time of the birth of the infants, these micro-organisms were isolated from 13 mothers and 10 infants. E. coli O112ab:B_{13} and O126:B_{16} were isolated most frequently.

During the first three months in the lives of the infants at home EPEC were isolated from cultures of rectal swabs from 4 of 21 mothers and from 18 of 63 infants from whom these micro-organisms had not been isolated while in the hospital. E. coli O55:B_5 was isolated most frequently.

In the hospital three mothers and seven infants had diarrhea, and EPEC were isolated from one mother and one infant.

The incidence of diarrhea in 28 of 30 infants at home occurred primarily among infants from whom EPEC serotypes had not been isolated during the first four days of life and from whose mothers these micro-organisms likewise had not been isolated during hospital confinement.

During the six-month period of this study, including the three months in the hospital and the first three months at home, a total of 900 persons were cultured, including 385 mothers, 386 infants, 114 personnel, and 15 siblings. Serotypes of EPEC were isolated from cultures of rectal swabs from 15.1% of the mothers, 12.7% of the infants, 1.8% of the personnel, and 33.3% of the siblings.

Serotypes associated with epidemic and sporadic cases of diarrhea were isolated from 64.9% of the positive subjects.

The diagnostic polyvalent E. coli antiserum prepared in this laboratory was essential for the preliminary detection of colonies of 11 serotypes of EPEC.

This study emphasizes the surprising prevalence of serotypes of EPEC among mothers, their infants, and siblings, the apparent ease with which they are acquired, and the unexpected incidence of asymptomatic intestinal carriers among mothers during hospital confinement.

Mrs. Mary C. Mahon assisted in obtaining information from the patient's records essential for compilation of the data.

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Fetomaternal Transfusion

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While the hemolytic process of erythroblastosis fetalis is by far the commonest cause of neonatal anemia, fetal anemia may result from a variety of causes.[1] Posthemorrhagic anemia of the newborn usually results from premature placenta separation or rupture of a placental blood vessel. A more subtle form of fetal hemorrhage was suggested by Weiner,[2] who postulated that transplacental bleeding into the maternal circulation could explain certain cases of nonhemolytic anemia of the newborn. In 1954 Chown[3] was able to prove this hypothesis in a case of neonatal anemia by demonstrating fetal cells in the maternal circulation. A number of definite instances of this syndrome have been reported since then,[1,4-9] as well as several probable cases.[10,11] Despite the frequency with which the condition is being reported, sufficient emphasis has not been given to the marked variability of the clinical signs and laboratory findings in these cases.

We have recently had the opportunity of studying two mothers and their infants. The infants had severe nonhemolytic anemia in the neonatal period. Fetomaternal transfusion was proven in each case by demonstrating in the maternal blood a markedly elevated level of fetal (alkali-resistant) hemoglobin and a minor population of antigenically dissimilar erythrocytes. One of the infants had a moderate *normochromic* anemia at birth and the clinical and laboratory findings of recent hemorrhage. The other infant appeared remarkably well compensated despite very severe anemia. Examination of his blood revealed a *hypochromic* microcytic picture suggesting chronic blood loss.

In both cases, the infant's blood was compatible with the mother from a transfusion standpoint and, although there were differences in blood groups or subgroups, no antibodies developed in the mothers. Studies of the disappearance of fetal hemoglobin from the maternal circulations after birth were performed. These studies supported the initial clinical and laboratory impressions concerning the time that the hemorrhages occurred in each case. They also suggested that fetal hemoglobin may be used as a biologic "tag" in determining red cell survival. In one case, the red cell life span, as measured by the disappearance of fetal hemoglobin from the mother's blood, was about 100 days.

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Dr. E. T. Blanch, Winchester, Mass., and Drs. R. T. Dowling and O. Z. Dashef, Woonsocket, R. I., provided clinical data in these cases and assisted in securing maternal blood specimens.

Methods

Alkali-resistant, or fetal, hemoglobin was determined by a minor modification of the method of Singer et al.,[12] in which the percentage of hemoglobin resistant to denaturation by $\frac{1}{12}$ N NaOH after 60 seconds is measured photoelectrically. The determinations were performed in duplicate, and the results were averaged. Blood counts, hemoglobin and hematocrit determinations, and red cell indices were determined by conventional methods. Agglutination studies were performed in test tubes using the appropriate potent saline antisera. The maternal sera were tested for antibodies against a panel of erythrocytes containing known antigens by saline, albumin, and indirect Coombs methods.

Report of Cases

CASE 1.—A white girl was born at term at a local hospital on March 19, 1958. The mother was a 33-year-old secundigravida, secundipara. The pregnancy was normal except for premature rupture of the membranes two days before delivery.

Labor was normal, terminating in an easy pelvic delivery. Estimated maternal blood loss was less than 300 cc., with no unusual bleeding. The placenta was said to be grossly normal. Birth weight was 3.3 kg. The infant appeared moderately pale; however, no meconium staining or yellowness of the vernix, a common indication of fetal distress, was observed. Hemoglobin on capillary blood was 12.1 gm/100 ml. (N=> 15 gm/100 ml.). At 8 hours of age the infant was pale but not jaundiced. The positive findings were a regular tachycardia of 170 per minute, rapid respirations, and a considerably enlarged liver extending 4 cm. below the costal margin and crossing the midline. The spleen was not palpable.

At the local hospital, it was found that the blood type of the mother was B, Rh-positive. The infant was Type O, Rh-positive, and direct Coombs test on his blood was negative. At 8 hours of age the infant's hemoglobin level was found to be 8.5 gm/100 ml., and it remained at this level on repeated testing. The white blood cell count was 17,900 per cubic millimeter, with polymorphonuclears 50% and lymphocytes 50%. Approximately 9 nucleated red cells per 1,000 red cells were seen. On the third day of life, the white blood cell count was 6,500 per cubic millimeter with marked decrease in the number of nucleated red cells. The urinalysis was negative. On the second day of life the meconium was slightly guaiac-positive, but thereafter it was negative. Chest x-rays showed no abnormalities. The infant remained in the local hospital 10 days and did very well. She never developed jaundice, and the liver gradually became smaller. At 12 days of age, she was seen in the hematology clinic. She was a pale but otherwise normal-appearing infant. The liver and spleen were not palpable.

Laboratory Data.—Hemoglobin, 8.2 gm/100 ml. (indices listed in Table 2). White blood cell count, 9,200 per cubic millimeter. Band cells, 4; polymorphonuclears, 40; eosinophils, 1; lymphocytes, 47; monocytes, 8. No nucleated red cells were seen. Platelets, 680,000 per cubic millimeter with many clumps. Red cells normochromic and normocytic.

Blood types: mother: Type B; CDe/CDe; indirect Coombs test, negative; infant: Type O; cDE/CDe; direct Coombs test, negative.

The maternal erythrocytes did not grossly react with anti-c or anti-E sera. However, when anti-c or anti-E sera were added to a suspension of maternal red cells a minor population of agglutinated cells was seen by microscopic examination. Precise quantitation of the minor population was performed by Dr. A. Richardson-Jones with his new method utilizing direct agglutination,[10] and the infant's cells were found to be 4.5%.

TABLE 1.—*Maternal Data*

Case	Days Post Partum	Maternal Hemoglobin, Gm/100 Ml.	Fetal Hemoglobin, %
1	12	14	6.5
	16	13.5	6.7
	22	13.2	5.9
	40	14.0	5.0
	57	14.6	3.5
	67	14.7	2.9
	82	14.3	2.4
	103	14.0	1.9
	120	14.2	1.7
2	0	10.0	9.3
	31	12.5	6.5
	51	13.0	2.5
	97	13.0	1.8

Fetal Hemoglobin: mother, 6.5% (normal < 2%); infant, 65% (normal=60%-90%). The upper limit of normal for an adult by the method used in our laboratory is 2.0%. This holds true in pregnancy as well.[14]

Specimens of maternal blood were tested frequently during the next three and one-half months, and these data are listed in Table 1. The curve of disappearance of fetal hemoglobin is drawn in Figure 1. The level of fetal hemoglobin was elevated above the normal maternal level for approximately 100 days. At 103 days post partum, Dr. Richardson-Jones found the minor population of infant's cells to be 0.10%.

The infant's blood was reexamined on three occasions. She developed microcytosis and hypochromia (Table 2), and, because of this oral ferrous sulfate was administered after the third month.

Starch-block electrophoresis of the maternal hemoglobin showed no abnormal hemoglobin and an A_2 fraction of 2.8%, thus excluding the hemoglobinopathies or thalassemia trait which may also

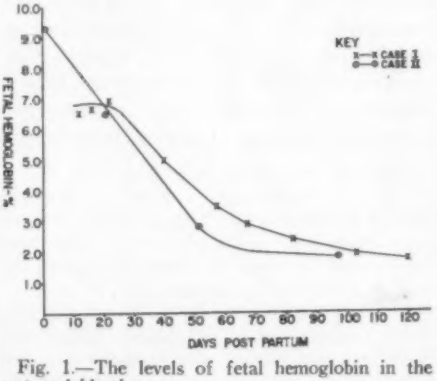

Fig. 1.—The levels of fetal hemoglobin in the maternal bloods.

TABLE 2.—*Blood Values on Infant (Case 1)*

Age, Days	Hemoglobin Gm/100 Ml.	Hematocrit, %	RBC, Million/ Cu. Ml.	Reticulocytes, %	MCV, Cu. μ	MCHb, $\mu\gamma$	MCHbC, %	Smear
12	8.2	29.0	3.31	2.2	87.5	24.8	28.2	Normochromic Normocytic
40	9.3	29.0	3.38	0.7	86.0	27.4	32.0	Normochromic Normocytic
67	10.5	32	4.10	0.5	78.0	25.6	32.6	Sl. hypochromia
102	9.7	30	4.35	0.3	69	22.5	32.2	Mod. hypochromia; mod. to marked aniso- and poikilocytosis; many microcytes

be associated with elevated fetal hemoglobin levels.[38] At no time could anti-c or anti-E antibodies be demonstrated in the maternal serum.

CASE 2.—A newborn white boy with severe anemia was referred to the Children's Hospital on Jan. 7, 1958. The mother was a 35-year-old gravida 6, para 6. Her previous pregnancies and children were normal. Although she was Rh-negative and her husband was Rh-positive, there was no suggestion of Rh sensitization. The pregnancy proceeded normally with confinement expected Jan. 8. About two weeks before delivery, the mother had a short episode of irregular uterine contractions. After this the fetus became less active. On Jan. 7, the fetal heart was considered to be weak and medical induction of labor was performed. Delivery was normal, and blood loss was not excessive. The amniotic fluid contained meconium, and the placenta was meconium-stained. There was a flat area of fresh hemorrhage, 3 in. in diameter, at the periphery of the placenta. This did not involve the marginal sinus. No infarction was present beneath the fresh clot.

The infant was said to appear normal at birth, and regular respirations were established in 3 minutes. At 4 hours of age, he was noted to be very pale. Hemoglobin was measured as 5.4 gm/100 ml. Coombs test was negative. He was then transferred to Children's Hospital.

Physical Examination.—He was a well-formed newborn, weighing 3.0 kg. Although the skin and mucous membranes were very pale, he was active and had a strong cry. There was no icterus. Respirations were 70 per minute. The apical heart rate was only 108 per minute. The heart and the liver were not enlarged. The tip of the spleen was palpated at the costal margin.

Laboratory Data.—Hemoglobin, 5.4 gm/100 ml; hematocrit, 20%; red blood cell count, 2.8×10^6 per cubic millimeter. Reticulocytes, 24.8%; MCV, 71.5μ; MCHb, 20.4$\mu\gamma$; MCHbC, 27%. White blood cell count, 27,600 per cubic millimeter; band cells, 5; polymorphonuclears, 68; lymphocytes, 24; normoblasts, 25/100 white blood cells.

On the peripheral smear there was a marked variation in the size and shape of the erythrocytes,

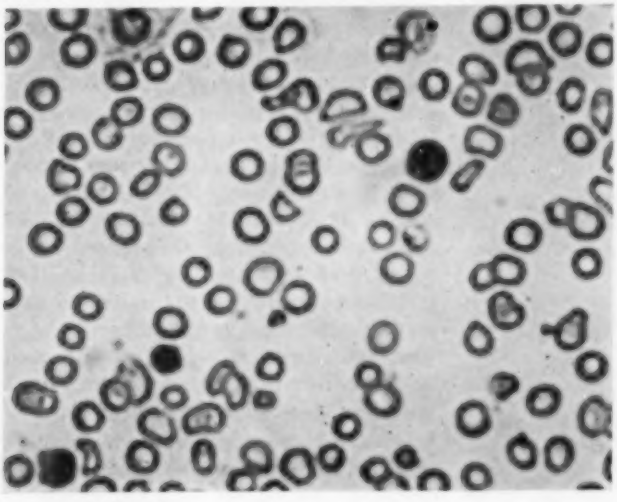

Fig. 2 (Case 2).—Peripheral smear of infant at 8 hours of age. Note microcytosis anisocytosis, hypochromia, and occasional "target cells."

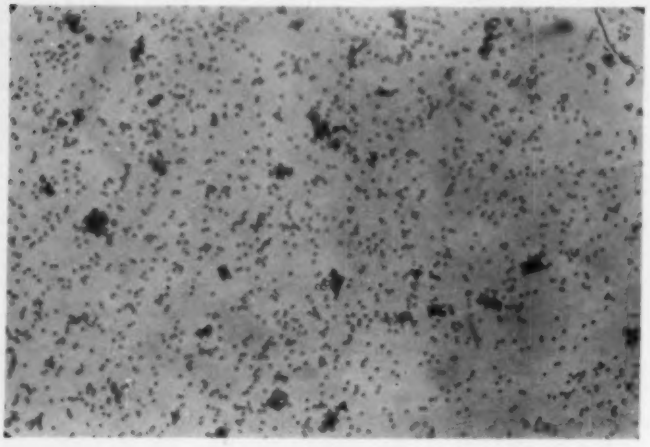

Fig. 3.—Saline suspension of maternal blood to which anti-N serum has been added. The groups of agglutinated erythrocytes are the transfused infant's cells; the free cells are maternal.

with many microcytes and large polychromatophilic cells. Hypochromia was prominent. Occasional target and stippled cells were noted. Platelets were normal (Fig. 2).

Bilirubin: Total, 2.0 mg. %; 1.5 mg. % indirect. Meconium was guaiac-negative. Blood types: mother: Type O; cde/cde M; indirect Coombs test, negative; infant: Type O; cde/cde MN; direct Coombs test, negative.

The mother's hemoglobin level was 10.0 gm. %. No nucleated red cells were seen on the peripheral blood smear.

When anti-N serum was added to a suspension of maternal erythrocytes small agglutinates were observed. Agglutinated cells were estimated to be present to the extent of 10% of the erythrocytes (Fig. 3). Fetal hemoglobin in maternal blood was 9.4% (normal $< 2\%$).

Shortly after admission, the infant was given a transfusion of 75 cc. of compatible packed red cells. There was marked improvement in his color. Post-transfusion hemoglobin level was 13.6 gm. %, and reticulocytes were elevated only to 13%. He was discharged on the third day of life in excellent condition. The subsequent course was satisfactory. Oral iron medication was administered after the third week of life. At 6 weeks, the hemoglobin level was 13.0 gm. %.

Maternal blood specimens were tested at 21, 51, and 97 days postpartum. The data are listed in Table 1, and the disappearance of fetal hemoglobin is charted in Figure 1. At no time could anti-N antibodies be demonstrated. Starch-block electrophoresis of maternal hemoglobin showed no abnormal hemoglobin and an A_2 fraction of 2.6%, excluding thalassemia trait.

Comment

Transplacental fetal hemorrhage was proven in these cases by the demonstration of a markedly elevated level of alkali-resistant hemoglobin and an antigenically dissimilar minor red cell population in the maternal blood. The fetal cells were compatible with the mother from a blood transfusion standpoint, and no antibodies developed in the maternal circulation in the months after pregnancy. Therefore, normal survival of the fetal cells could be anticipated in these cases.

Experimentally, the life span of the fetal erythrocyte transfused into adults has been estimated by two methods. Using the Ashby technique, Seelemann [16] and Mollison [17] concluded that there was no appreciable difference in the survival of fetal and adult erythrocytes. However, using radioactive-chromium tagging, Hollingsworth [18] demonstrated an apparent shortening of fetal erythrocyte survival. The half-life of fetal cells, as determined by chromium-51, was only 2/3 of the normal adult value. The probable mechanism to explain this discrepancy was described by Siederman et al.,[19] who found that in vitro chromium-51 could be eluted from fetal hemoglobin three times faster than from adult hemoglobin.

In Case 1, the time that the hemorrhage occurred can be fixed as shortly before

delivery, probably during labor. At birth, no meconium staining such as usually accompanies fetal distress was observed and the infant had tachycardia and transient hepatomegaly. The hemoglobin initially was 12.1 gm/100 ml., but eight hours later it had fallen to 8.5 gm/100 ml. This probably indicates that at birth the infant was in the relatively hemoconcentrated phase which is seen shortly after acute hemorrhage.

About 100 days elapsed between delivery and the time that the level of the fetal hemoglobin in the maternal blood had fallen to normal adult values. These data support the Ashby studies and indicate a normal survival of the transfused fetal erythrocyte. Two assumptions are implicit in this conclusion. First, the maternal contribution of the alkali-resistant fraction of hemoglobin does not change significantly during the months after delivery. To our knowledge no systematic serial study of this has been performed. The second assumption is that fetal hemoglobin is not reutilized per se after being liberated from the senescent red cell. On the basis of experimental work,[20] it is generally believed that the globin is broken down completely to amino acids. These enter the protein metabolic pool before new globin is synthesized.[21]

In Case 2, the infant was well compensated to severe anemia at birth. The red cells were microcytic and hypochromic with anisocytosis, a picture seen in iron deficiency or chronic blood loss. The blood volume of a pregnant woman at term is about 10% of the total body weight. The mother in Case 2 weighed 54 kg. at term. Her total circulating hemoglobin may be calculated to be approximately 540 gm. (54 kg.$\times$10%$\times$10 gm. %). Since about 7.5% of this hemoglobin was alkali-resistant hemoglobin originating from the fetus, the amazing quantity of 40 gm. of the infant's alkali-resistant hemoglobin was present in the maternal circulation at term. This represents about 335 cc. of normal neonatal blood, an amount greater than the entire blood volume of an average newborn. A hemorrhage of such magnitude would have had to occur over an extended period of time to be compatible with fetal survival.

Only a limited number of post-partum samples of maternal blood were tested in Case 2; however, nearly 90% of the exogenous alkali-resistant hemoglobin present in the maternal circulation at the time of delivery had disappeared 51 days post partum. In the absence of accelerated destruction due to hemolysis, this too suggests that a major portion of the hemorrhage had occurred a considerable while prior to delivery. In cases of this syndrome, the clinical and laboratory findings may vary widely, depending upon the duration and magnitude of blood loss and the time that it occurs. Undoubtedly, unrecognized cases occur with relatively minor blood loss. Conceivably, this blood loss could be important in some cases of infantile iron-deficiency anemia. If the hemorrhage is rapid and massive the infant either dies in utero or exhibits signs of severe posthemorrhagic shock at birth. If the infant's cardiovascular system is able to adapt successfully to the degree of blood loss, anemia and pallor are the cardinal signs. Jaundice is absent. Significant enlargement of the liver and spleen is not present unless it is due to congestive heart failure secondary to the anemia.

In an affected infant's blood recticulocytosis and increased numbers of nucleated red cells are usually seen, indicating an active marrow response to hemorrhage. If the hemorrhage is recent, the platelet count may also be elevated. Direct Coombs test on the infant's red cells is negative and hyperbilirubinemia is unusual, thus differentiating this syndrome from erythroblastosis. The erythrocyte morphology may vary considerably. First, as in Case 2 reported here, a congenital hypochromic microcytic anemia may be observed if chronic blood loss and depletion of fetal iron stores has occurred, but if the bleeding has occurred shortly before delivery, a normochromic normocytic anemia will be found.

The exact mechanism by which the fetal blood enters the maternal circulation in

these cases is uncertain. Chown observed a probable site of hemorrhage in the placenta of one of his cases[3]; however, in most of the reports, the placenta has been said to be grossly normal. The placenta is an organ with limited life span, and vascular occlusion and infarction are common findings at the time of delivery. Kline[22] examined placentas from various stages of gestation and demonstrated numerous microscopic breaks in the placental barrier, with minor fetal hemorrhage into the maternal intervillous spaces. The breaks were considered to be secondary to vascular occlusion and infarction of villae. They were rapidly sealed off by clot formation with subsequent organization. This type of mechanism, with continuing ooze, may well have been operative in Case 2. It may be assumed that a pressure gradient existed that allowed protracted flow from the fetus to the mother. In Case 1, with abrupt acute hemorrhage, probably occurring during labor, the force of the contracting uterus may well have played an important part in the formation of a fetomaternal vascular fistula.

In cases of this syndrome, the mother usually experiences no symptoms as a result of the transfusion from her baby. However, instances have been reported in which the infant's blood was incompatible with the mother's and a transfusion reaction characterized by fever and chills or hyperbilirubinemia occurred.[3,5] Cases of Rh sensitization in mothers following gross fetomaternal transfusion have also been documented.[5]

The treatment of the infants in these cases is simple transfusion with packed or sedimented cells if the anemia is of severe degree or if the infant shows signs of distress. Because of the possibility of protracted hemorrhage, and also because the loss of any blood from the fetus may strain the already precarious iron balance of the infant, supplemental oral iron medication is indicated in the recovery stage.

Summary

Two cases of proven fetomaternal transfusion are described. The marked variability of the clinical signs and laboratory findings in these cases is emphasized. Depending upon the time that the hemorrhage occurs and upon its magnitude, the newborn may have findings ranging from acute posthemorrhagic shock (with relatively little apparent hemoglobin loss) to severe but well-compensated chronic anemia. In one of the cases reported here, by utilizing fetal hemoglobin as a biologic "tag," the survival of the transfused fetal erythrocyte was determined to be about 100 days.

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Attempted Abortion with Aminopterin (4-Amino-Pteroylglutamic Acid)

Malformations of the Child

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Pteroylglutamic acid (PGA, folic acid) plays an important role in many biological reactions, and PGA deficiency may lead to death of mammalian embryos. Nelson and Evans [1] demonstrated reproductive failure in rats kept on a PGA-deficient diet containing succinylsulfathiazole and a crude PGA antagonist. Thiersch and Philips [2] induced fetal death in rodents kept on an adequate diet by administration of aminopterin (4-amino-pteroylglutamic acid), a powerful PGA antagonist. Later Giroud and Lefebvres-Boisselot [3] and Nelson, Asling, and Evans [4] produced multiple congenital abnormalities by maternal PGA deficiencies during gestation.

Aminopterin has also been used to induce therapeutic abortions in women with tuberculosis or cancer. In 10 out of 12 cases reported by Thiersch [5] oral doses of 6 to 12 mg. aminopterin induced fetal death in the first trimester of pregnancy, followed by spontaneous delivery of the products of conception. The doses lethal to the embryo had only mild effects on the blood of the mothers. In the younger fetuses depression of hematopoiesis and necrosis of the liver, adrenals, and intestinal epithelia were observed. In three older fetuses the drug failed to produce immediate death but induced congenital malformations, such as hydrocephalus, meningoencephalocele, or harelip and cleft palate. In 1956, Thiersch [6] reported results on 12 additional cases treated with aminopterin. Six of these patients failed to abort, and the fetus of one of them went to term after therapeutic abortion had been attempted in the (estimated) first month of pregnancy with 12 mg. of aminopterin. The infant had anencephaly and lived for 12 days. Meltzer [7] reported the case of a 25-year-old woman who attempted abortion with aminopterin in the second month of pregnancy. Forty tablets of 0.5 mg. were taken over a period of several weeks. Bleeding was provoked, but abortion failed to occur. The patient continued to bleed intermittently to about the 24th week of her pregnancy, and after that time the prenatal course was uneventful. Following a normal labor, a living infant weighing 3,203 gm. was delivered. The child had a left talipes equinovarus and multiple skull anomalies, such as partial craniosynostosis and "lückenschädel."

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From the Children's Hospital Research Foundation and the Laboratory for the Study of Maternal and Infant Health of the Departments of Obstetrics and Pediatrics, University of Cincinnati College of Medicine.

Report of a Case

We have had an opportunity to observe a child whose mother had also taken aminopterin during early pregnancy without aborting. This 27-year-old mother of six normal living children visited the dermatology clinic of the Cincinnati General Hospital on Dec. 21, 1956. She complained of ulcerations of the lips of four days' duration and of a maculopapular pruritic rash covering the body, with the exception of the face, palms, and soles. Her last menstrual period had started on Sept. 30, 1956, and lasted seven days. Believing that she was pregnant and wanting to abort, she had purchased aminopterin tablets from her druggist and had ingested a total of 24 0.5 mg. tablets at a

rate of two tablets per day from Dec. 8 until Dec. 19. She discontinued the medication on that day because of the appearance of the rash.

The patient was admitted to the Cincinnati General Hospital on the day of her visit to the clinic, Dec. 21, 1956, with the diagnosis of "aminopterin poisoning and erythema multiforme." She was admitted for assessment of her pregnancy, for evaluation of her blood picture, and for treatment, if necessary. A bone marrow sample taken on the day of admission showed "recovery from toxic suppression" (Dr. C. E. Kiely). Repeated peripheral blood smears were normal. A Friedman test done on Dec. 28 was positive. Clinical examination confirmed a two to three-month pregnancy with no evidence of imminent or threatened abortion.

Treatment consisted of one dose of 30 mg. of PGA per os and 3 mg. of citrovorum factor injected daily intramuscularly for a total of 10 days. The lesions cleared up rapidly. Because of the known teratogenic effects of aminopterin, interruption of pregnancy was considered at that time. Since there is no legal basis in Ohio for therapeutic abortion unless the mother's health is seriously threatened, the pregnancy was not terminated, and the patient was discharged on Jan. 4, 1957. Delivery was expected approximately on July 7.

The remainder of her pregnancy was essentially uneventful, and at no time was there any evidence of threatened abortion. On July 9, 1957 (13 days before delivery), an anteroposterior roentgenogram of the mother's abdomen was obtained: "An intrauterine pregnancy is evident. The uterus appears large in porportion to the size of the fetus, suggesting hydramnios. The fetus shows a strikingly abnormal finding: failure of ossification of a large segment of the membranous calvarium. One of the lower extremities of the fetus is obviously extended, but the other extremities show normal flexion, so that the fetus is not believed to be edematous. The skeleton of the hands and feet are not well shown. No epiphyses are seen about the knees. The fetus appears to be in about the 8th month of gestation, occiput to the left. The placenta is not visible. Impression: Hydramnios; abnormal delay in ossification of the fetal calvarium, etiology undetermined. One should consider cleido-cranial dysostosis, hypophosphatasia, and osteogenesis imperfecta (although no other bony abnormalities are evident)" (Dr. B. Felson).

The mother's entire weight gain during pregnancy was 10 lb. Delivery was spontaneous, with occipital presentation on July 22, 1957, approximately two weeks after the calculated date.

The child's respiratory effort was spontaneous but irregular, the heart rate was slow, and the extremities, held in flexion, were blue. The child responded with a grimace to a nasal catheter, and resuscitation was not required. A score of 5 on the Apgar scale* was given to the infant, who was immediately transferred to an Isolette in the premature nursery. The birth weight was only 1,280 gm., although delivery was about two weeks overdue. A crown-heel length of 36 cm. was proportionate to the weight. The greatest circumference of the head was 32 cm., which was relatively large for the weight and size of the infant.

Fig. 1.—Photograph of child, showing large head, small mandible, high philtrum, low-set ears, vertical skinfolds of neck, and abnormal position of extremities.

The head showed numerous abnormalities (Fig. 1). The spherical skull, at first mistaken for a hydrocephalus, was combined with a very small face. There was a moderate amount of light brown hair extending from the superior orbital ridge, over the forehead and crown to the tip of the ears, and posteriorly to the neck. The skull was soft and felt like a "rubber balloon filled with

water," somewhat reminiscent of the soft skull in the severe type of osteogenesis imperfecta, hypophosphatasia, or craniocleidal dysostosis. Rudimentary frontal and temporal bones, widely separated, could be felt. There was a total absence of ossification of the parietal bones, but the occipital bone was of normal size and consistency.

The eyes were far apart; the distance between the inner canthi was markedly increased, and the nasal bridge was broad (Fig. 1). The eyeballs seemed somewhat prominent because of underdevelopment of the supraorbital ridges. There were a few eyelashes but no eyebrows. Otherwise the eyes appeared normal. The nose was flat, and

Fig. 2.—Lateral view of child, showing abnormal ears.

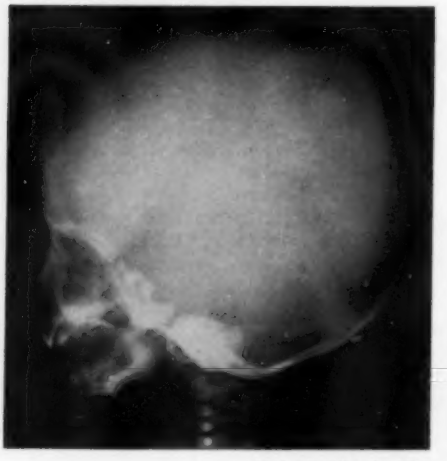
the nostrils were patent. The external ears were very low set and seemed retained near the midline. Instead of being directed laterad, they were turned laterad and downward. The mandible was small and the position of the ears characteristic of cases of severe micrognathia, with retention of the ears near the primitive hyomandibular cleft position.[8] The shape of the pinna was most unusual (Fig. 2). The anterosuperior portion of the helix and the crus helicis were present, but the entire posteroinferior part of the helix and the lobule were absent. The anthelix, antitragus, and

Fig. 3.—Posterior palate cleft.

tragus were developed, and the external canal, though very narrow, was apparently patent. A speculum could not be introduced. The mouth was very small and the upper lip and the philtrum unusually high (Fig. 1). A posterior palate cleft was present, but because of the small opening of the mouth it was difficult to visualize and photograph it (Fig. 3). Vertical skin folds, reminiscent of a pterygium colli, were seen on the sides of the neck (Fig. 1).

In contrast to the large head, the body was small. The chest was bell-shaped, and its cir-

Fig. 4.—Roentgenogram of skull, showing incomplete ossification of calvarium.



cumference at the level of the nipples was 20.5 cm. Percussion and auscultation were normal. The abdomen was markedly protruding, and the liver was palpable 2 cm. below the right costal margin in the midclavicular line. The pubic area was broad and exposed. The external genitalia were female, the labia minora being represented by two small triangular tags of tissue. The hymen was present.

The extremities showed no gross anatomical malformations, but they assumed abnormal positions (Fig. 1). The hands were large and long in comparison with the forearms and the upper arms. The fingers were slender and moved well. The lower extremities were held extended, and the feet turned in, but there were no contractures. The joints were unusually mobile, and there was general laxity of the ligaments. The gluteal and inguinal skin folds were deep, and there were many other abnormal skin creases on the arms, on the legs, and around the nipples.

Laboratory Findings.—Hemoglobin, 21.9 gm. per 100 ml.; erythrocytes, 6,290,000 per cubic millimeter; reticulocytes, 10.8% of red cells; leukocytes, 27,500 per cubic millimeter with a differential count of 45% neutrophils, 44% lymphocytes, 9% monocytes, and 2% eosinophils; platelets, 220,150 per cubic millimeter. The chromosomal pattern was female. Serology of the mother was negative.

X-Ray Findings.—On July 22 and 23, 1957, x-ray findings of the infant included full-body film and views of the skull, hip, and lower extremities. "The incomplete ossification of the calvarium is again apparent [Fig. 4]. There is ossification only of the occipital bone and of a relatively small portion of each frontal bone. No other abnormalities are seen in the skull, thorax, abdomen, or spine, except that the liver appears small. There is retarded ossification of the pubis and ischium and bilateral dislocation of the hips is noted [Fig. 5]. The hands and feet appear clubbed. The radii are relatively short. Impression: Multiple congenital anomalies, including markedly delayed ossification of calvarium, pubis, and ischium; bilateral dislocation of hips; clubbing and synostosis of hands and feet; shortened radii" (Dr. B. Felson).

Family History.—The infant described above was the only abnormal child in this family. There were five brothers and one sister, all mentally and physically normal. The mother had had a spontaneous abortion in the third month of gestation in 1950. The parents were physically and mentally normal and not consanguineous. The mother's parents were deaf, but the defect of her father was attributed to an accident and that of her mother to spinal meningitis. The mother's hearing was good, and her siblings and their children were normal. On the father's side a

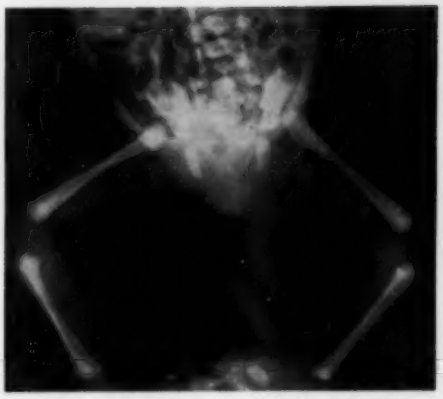


Fig. 5.—Roentgenogram of pelvis and of extremities. Retarded ossification of the pubis and ischium and bilateral dislocation of hips can be seen.

history of 59 relatives was obtained. With the exception of one cousin, who had been born blind but with normal intelligence, no congenital anomalies occurred on the paternal, side. No harelip, cleft palate, ear anomalies, crippling disorders, or mental retardation were reported on either side of the family.

Clinical Course.—The infant was kept in an Isolette under high humidity. Respirations were regular and not labored but of small amplitude. The color was good but the cry weak and high-pitched. The child was moderately active, yet the over-all impression was that she was in poor condition, even though no specific system could be incriminated for her general weakness. On the second day the respirations became suddenly labored, and the child had a few episodes of apnea and died at 29 hours of age, during one such episode. No satisfactory explanation could be found clinically for the cause of death.

Autopsy.—The dissociation between the well-documented gestational age and body maturity of this child was further confirmed by the autopsy findings. The bony calvarium was markedly reduced in size to two very small widely separated frontal bones (4×3 cm.) and two rudiments of temporal bones (1×1 cm.). The parietal bones were still in the membranous state, but the occipital bone was totally ossified. The weight of the brain was 350 gm., which is average for a normal full-term child but large for this child of 1,280 gm. It appeared somewhat rounder than normal but had no gross external abnormalities. Microscopically, the cerebral cortex showed no acute or chronic degenerative changes and appeared more mature than is usual in the full-term newborn baby. The lungs and kidneys shared in this mature appearance, but their weight was not excessive in

regard to the total body weight. The lungs showed infiltration with round cells, compatible with the diagnosis of chronic interstitial pneumonitis. The ovaries, adrenals, and cerebellar cortex showed a degree of immaturity in keeping with the small body weight. There was a defect in the right leaf of the diaphragm 1 cm. in diameter, through which three nubbins of liver herniated, and a Meckel's diverticulum. All other organs, except the thymus, which was extremely small, were of normal weight, size, configuration, and position (Dr. A. J. McAdams).

Comment

The birth of a child with congenital malformations following attempted abortion raises the question of causal relationship of the two events. It is difficult, in a single case, to establish with certainty that the abortifacient damaged the embryo, but animal experiments with teratogenic agents have demonstrated that malformations arise in a border zone between fetal death and normality, and there exists the possibility that unsuccessful attempts to induce abortions may damage the embryo without terminating pregnancy. Whether such abortive attempts are actually an important cause of congenital malformations in man has often been discussed, but there is little evidence that it is so. Whitehouse and McKeown,[10] investigating the significance of attempted abortion in the etiology of anencephaly, came to negative conclusions. In the context of this question the case presented above has several features of interest. PGA deficiency created in pregnant rodents experimentally with the aid of antagonists represents a rather specific teratogenic method. Under certain conditions 100% of the young can be rendered abnormal. To obtain malformations with certainty, the intensity of the PGA deficiency must be controlled and the depletion period timed. Instituting the deficiency too early in gestation invariably results in fetal death, while delaying the deficiency beyond the period of organogenesis results in normal young.[4]

The antagonist, aminopterin, ingested by the mother of the child described above, is known to induce abortion in women if administered in very early stages of pregnancy and to produce malformations if given in insufficient amounts in more advanced stages. In one series,[5] 3 of 12 attempted abortions failed, and after surgical intervention abnormal fetuses were found. Malformations were observed in the fetuses of three women who had received 10, 10, and 15 mg., respectively, of aminopterin, beginning on days 40, 49, and 54, respectively, of pregnancy. Under the assumption that fertilization took place about two weeks after the last menstrual period, the ingestion of the drug began in our case on Day 55 and lasted to Day 66 of (estimated) fertilization age, the total dose amounting to 12 mg. Thus the dose was of the same magnitude as that reported in the cases of Thiersch,[5] but the delay was probably even greater. The malformations of the child were less severe than those in the series of Thiersch, in which meningoencephalocele, hydrocephalus, and harelip with cleft palate occurred. Can the malformations of this child be attributed to the agent applied from the 10th to the 12th week of gestation, when the embryo was probably 8 to 10 weeks old?

It has been emphasized that the much-practiced method of retrospective timing of the origin of congenital malformations is fraught with danger.[11,12] However, it is possible sometimes to *exclude* the possibility of a causal connection between an external event and the formation of a congenital defect if the incriminated incident occurred *after* the formative period of the affected organ. For instance, a spina bifida apertura with an open neural plate cannot be attributed to an incident in the third month of pregnancy, since the neural tube is normally closed in an embryo 4 weeks old. But many congenital malformations can be explained by retarded or abnormal growth in early or in later periods of gestation, and retrospective timing of the deviation from the normal becomes very hazardous.

In the case described above the administration of a teratogenic drug was

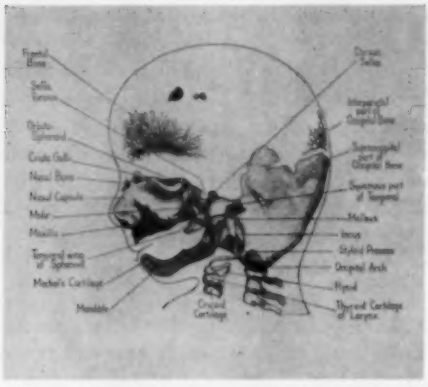


Fig. 6.—Skull of a human embryo of about nine weeks (38-40 mm.). Cartilage is shown in gray wash; the locations of the endochondral ossification centers which have appeared at this age are indicated by superimposed stippling; the lace-like trabeculae of young intramembranous bone are shown in black. Courtesy of Bradley M. Patten, "Human Embryology," Ed. 2, New York, The Blakiston Company, (division of McGraw-Hill Book Company, Inc.) 1953.

limited to a rather well-defined time of gestation. Before one attributes the malformations of the child to the ingestion of the drug, one has to ask if they could have been caused by the drug or if they indicate interference with development before its administration.

Many malformations of this child, such as those of the extremities, do not lend themselves to chronological analysis, since they could be attributed to early or to late deformation.

If one compares the skull of this child (Fig. 4) and that of a 9-week embryo (Fig. 6), one finds that the membrane bones which constitute the sides and the roof of the cranium are absent in both. The base of the skull can be seen in the 9-week embryo in cartilaginous form, and ossification has begun in some of the facial and long bones and in the clavicle. Ossification of the frontal bone begins in the 9th week and that of the parietal bones in the 10th.[9] It seems as if the drug administered during the 9th and 10th weeks had interfered with ossification of the membrane bones which normally begins at that time but not with ossification of those bones which normally chondrify or ossify before nine weeks.

According to Fulton,[13] the threshold period of palatal closure lies between 47 and 49 days of ovulation age. In his study touching of the converging maxillary processes at any place along the palatal plane was considered as "closure." However, at this stage the palate is not completely sealed, as the palate closes in an X configuration, first at a point distal to the anterior end, continuing backward through the middle half, then closing through the anterior fourth, and, finally, through the posterior fourth. In Fulton's series the palate was still open in one embryo of 53 days of ovulation age. If one takes into consideration individual variations and the uncertainties of establishing ovulation age by menstrual history, one cannot exclude the possibility that the final closure of the posterior portion of the palate was inhibited in this child by the drug administered from the 56th day on.

The development of the auricle in the human embryo was described in detail by Streeter.[14] In very young embryos with undeveloped mandibles the areas involved in the formation of the external ear are located very low and near the midline. The auricular hillocks develop around the first gill clefts, which lie between the mandibular and hyoid arches. As the mandible grows the auricular area is displaced upward and backward, but in some cases of agnathia and micrognathia the ears maintain more or less their low near-midline position (synotia). In embryos 16-18 mm. long (about the seventh week) the auricle assumes still a horizontal position with the mandibular portion located above the hyoid portion. As the ear moves upward and backward the mandibular bar becomes the anterior and the hyoid bar, the posterior part of the auricle. The tragus and the crus helicis develop from the anterior, mandibular, bar; the helix, anthelix, antitragus, and lobulus from the posterior, hyoid, bar. Differentiation of the hyoid bar into helix and anthelix, which begins in

embryos of about 50 mm. crown-rump length,[14] takes place early in the third month.

The auricles of this child show some arrest of ascent and are located low and near the midline, as seen in some cases of micrognathia[9,14] (Fig. 7). Since the ascent of the auricle is a gradual one, it is not possible to assign the midposition of the auricles of this child to any definite embryological stage or gestational age. The absence of a large part of the helix and of the lobe could be attributed to faulty differentiation of the hyoid bar into helix and anthelix, which takes place in the first half of the third month. The possibility that the deformity of the ear was brought on by ingestion of aminopterin between Days 56 and 68 cannot be excluded.

The family history is negative for cranial dysostosis, cleft palate, auricular deformities, and crippling diseases. Although this does not rule out the possibility that abnormal genetic factors played a role in the malformations of this child, there is no proof that they did.

Since aminopterin and other folic acid antagonists have been proven to be teratogenic in man and in experimental animals and since some of the malformations found in this child can be explained by interference with normal embryonic development from the 10th to 12th weeks of gestation, when the embryo was probably 8 to 10 weeks old, it seems reasonable to assume that the anomalies of the child described can be attributed to unsuccessful abortion attempted with aminopterin.

Fig. 7.—Micrognathia with external ears remaining in the primitive hyomandibular cleft position. Courtesy of Bradley M. Patten, "Human Embryology," Ed. 2, New York, The Blakiston Company, (division of McGraw-Hill Book Company, Inc.) 1953.

Summary

Multiple congenital malformations are described in a child whose mother attempted abortion with aminopterin (4-amino-pteroylglutamic acid) in the 10th week of gestation. The child showed intrauterine growth retardation, cranial dysostosis, posterior palate cleft, mandibular hypoplasia, and anomalies of the ears. It seems probable that this unusual syndrome was caused by ingestion of the drug, which interfered with normal fetal development in the third month of gestation.

Children's Hospital (*29*).

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Calcification of Intervertebral Disks in Children

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Calcification of intervertebral disks in children is a curious clinical and radiological entity. The purpose of this paper is to report two children with intervertebral disk calcification. The clinical manifestation, etiology, and pathological physiology are discussed, including differences in children as compared with adults.

Nineteen cases have been reported in children. The first, in 1924, was by Báron.[1] Later, in 1954, Silverman reviewed eight and reported seven other cases.[13] In 1954 two more cases were reported,[12,17] and in 1957, two additional cases.[6,15]

Report of Cases

CASE 1.—An 8-year-old Negro boy was admitted to Duke University Hospital. He had been born at this hospital of an uncomplicated "term" pregnancy, weighing 1,855 gm. Labor was short; low forceps were used in delivery. He remained in the premature nursery for three weeks and weighed 2,240 gm. on discharge, at which time the physical examination was negative. Blood from the mother and cord blood both gave a negative serologic test for syphilis.

At home he received a 20 Cal. per ounce formula, and development was reported as normal, until at one year of age he was noted to have bilateral cataracts, presumably congenital in origin. Cataract extraction was attempted at 4 years of age. Otherwise he remained in good health until two weeks prior to admission, at which time he had attempted to lift the side of a trailer, without apparent consequence. The next day he fell while playing, with no evidence of trauma. He first noted some low back pains three days later, associated with mild recurrent cramping abdominal pain. The backache became severer and intermittent, and he then started to complain also of stiffness of the back. He had no headache, fever, nuchal rigidity, anorexia, nausea, or vomiting. The back pain changed to a constant aching, with episodes of severe sharp pain at the level of the dorsolumbar spine junction but with no radiation; these episodes occurred when the patient moved or was moved. Three days prior to admission he was admitted to another hospital and then transferred to this hospital.

At the first hospital, the physical examination was reported as negative except for "marked arching of the lumbar spine." Routine analysis of the blood, urine, and spinal fluid were all reported as normal.

On admission to The Duke University Hospital he was noted to be a well-developed slender Negro boy with a blood pressure of 112/68 and a rectal temperature of 37.7 C (99.9 F). His back was markedly arched in an exaggerated lordotic posture, with a severe dorsolumbar paravertebral muscle spasm. There was no evidence of trauma over his trunk. The child was in no distress while lying undisturbed. However, he experienced excruciating pain when examined or moved. Eye examination revealed a capsular cataract of the left eye and an operative coloboma of the right eye. There was a slight injection of the pharyngeal wall, and the neck was supple. The lungs, heart, abdomen, genitalia, and extremities were normal. The neurological examination revealed normal superficial and deep tendon reflexes. There was no motor weakness or sensory deficit. Kernig's and Brudzinski's signs were present.

Accessory Clinical Findings.—The hemoglobin, hematocrit, white blood cell and differential count, erythrocyte sedimentation rate, total protein and albumin-globulin ratio, fasting blood sugar, and total cholesterol determinations were all normal. The complete urinalysis and tests for alkaptonuria were negative. The serum calcium ranged from 10.0 to 10.9 mg.; the phosphorous, from 4.0 to 5.9 mg., and the alkaline phosphatase, from 2.7 to 5.0 Bodansky units per 100 ml. of blood. These tests were subsequently repeated at intervals during the follow-up period and remained normal. The C-reactive protein, sickle-cell preparation, serological test for syphilis, Brucella agglutination test, and skin tests for histoplasmosis, blastomycosis, coccidioidomycosis, brucellosis, as well as old tuberculin (from 1:10,000 to 1:100), were all negative. A lumbar puncture revealed normal pressure; the cerebrospinal fluid was clear and colorless, with 2 mononuclear cells per cubic millimeter, and Pandy's test was negative. Spinal fluid sugar was 69 mg.; protein, 28 mg. per 100 ml., and chloride,

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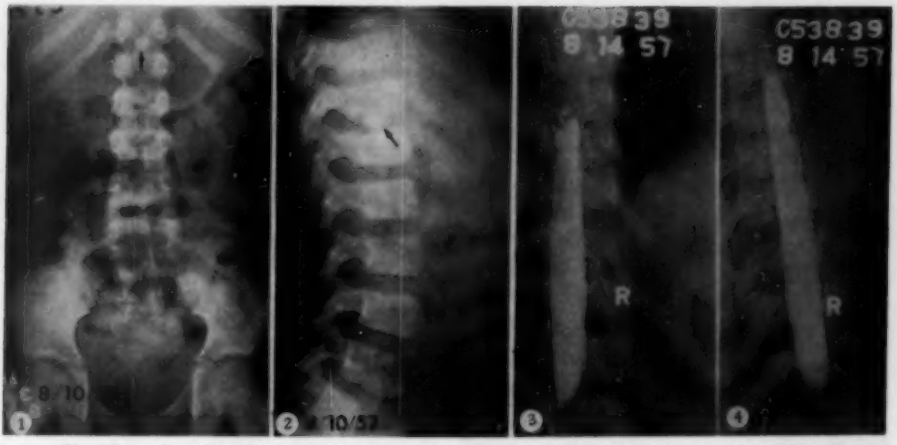

Figs. 1-4 (Case 1).—Roentgenograms and myelograms of the dorsolumbar region. Arrows point to the calcified nucleus pulposus at T-12.

127.0 mEq. per liter. A culture of the fluid was negative.

Roentgenograms of the chest were normal, whereas studies of the spine revealed a calcification in the nucleus pulposus at T-12 intervertebral disk (Figs. 1 and 2). Dorsolumbar myelography revealed the contrast media to pass without obstruction throughout the lumbar and thoracic canal (Figs. 3 and 4). No defect was seen at T-12 to L-1 level. ECG was suggestive of right and left ventricular hypertrophy but without additional confirmatory findings.

Course of Illness.—The patient was kept in the hospital on bed rest and received acetylsalicylic acid (0.32 gm. every 4 hours if necessary for pain). His course did not change for days. He was then placed in a body cast and discharged on the seventh hospital day, to be followed in the outpatient department. The cast was removed after six weeks. X-ray of the spine then revealed diminution of the calcified area (Fig. 5). He was gradually allowed more movements, and he was seen three weeks later, after the removal of the cast. At this time, the x-ray films revealed marked reduction in the size of the calcified area (Figs. 6 and 7). He had no complaints of pain while he was in the cast and remained completely asymptomatic afterward.

Case 2.—A 13-year-old Negro boy visited the outpatient department after having had intermittent back pain for three months. The pain was preceded by lifting a heavy object a few days earlier, but it was eased by bed rest and symptomatic treatment at home, as prescribed by his local physician.

The backache recurred after he was hit on the back by another boy one week prior to his visit. The pain was confined to the upper dorsal region and was nonradiating. The physical examination was completely unremarkable. The accessory clinical findings revealed hemoglobin of 15.5 gm. and white blood cell count of 14,850, with 65%

Figs. 5-7 (Case 1).—Roentgenograms showing the diminution in size of the calcified area.

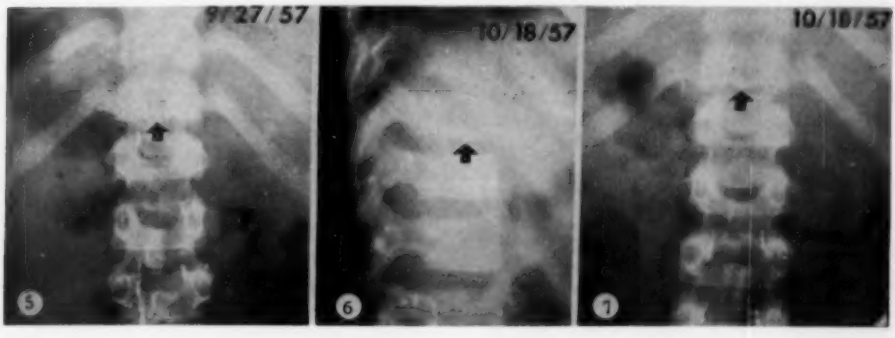

Fig. 8 (Case 2).—Roentgenogram of thoracic spine. Arrows point to calcification in intervertebral disks, T-5 and T-6.

polymorphonuclear cells, 3% eosinophils, 2% monocytes, and 30% lymphocytes. Urinalysis and sickle-cell preparation were negative. The x-ray of the spine showed calcification of intervertebral disks T-5 and T-6 (Fig. 8). The chest x-ray was not remarkable. A follow-up was not obtained.

Comment

I. Anatomy and Physiology of Intervertebral Disk.—The structure and physiological significance of the intervertebral disks have been carefully reviewed elsewhere,[4,7,11] but they are briefly outlined below. An intervertebral disk consists of three distinct parts: the nucleus pulposus, the annulus fibrosus, and the cartilaginous plates (or limiting plates). The plates are of hyaline cartilage and firmly adhere to the intervertebral surfaces adjoining the vertebral bodies. The annulus fibrosus binds the adjacent vertebral bodies and constitutes an envelope surrounding the nucleus pulposus like a capsule. The nucleus pulposus is a semigelatinous structure and is "encapsulated" by the cartilaginous plates and the annulus fibrosus.

In early age, the nucleus pulposus is rich in blood supply,[2] which markedly diminishes toward the third decade of life.[4] The nucleus pulposus consists of few cells, poorly differentiated collagenous fibers, and an amorphous substance. The amorphous substance is composed of polysaccharides, a protein component attached to the polysaccharide, and water. With increasing age, the water content and elasticity diminish and the fibrous element becomes more prominent.[3,14] Sylvén et al., 1951,[15] using electron microscopy, described the interfibrillar matrix as "a three dimensional lattice gel system with a regular molecular pattern."

The intervertebral disk is a unit which "transmits, modifies and evenly distributes the force of strain."[4] The annulus, composed of concentric rings of fibrous cartilage, is compressible and elastic. The nucleus, on the other hand, composed of semiliquid mucoid material is noncompressible. The nucleus, together with the annulus, functions as a "hydraulic ball bearing."[3,9] The nucleus occupies an eccentric position in the middle of the disk, being localized more anteriorly in the cervical and lumbar vertebrae.[10] Any movement of the spinal column is accompanied by a shift of forces in each nucleus pulposus. Flexion of the spinal column causes an anterior compression of the nucleus which results in displacing it posteriorly. Anterior displacement occurs during extension.[4] The nuclei pulposi are displaced (or situated) in a direction opposite to that assumed by the curvatures of the spine, whether these curvatures are transient as during movement or permanent as in normal and pathological posture (curvatures).

II. Calcification of Intervertebral Disks.

Calcification may occur in the cartilaginous plates, the annulus, or the nucleus pulposus. Sandström, 1951,[10] maintains that calcification of the plates and the annulus

is generally permanent, while calcification of the nucleus may or may not be reversible. Calcification of the annulus is reported to be 10 times as frequent as that of the nucleus.[12]

In adults, the calcification usually involves the annulus and the plates, with occasional involvement of the whole disk. In general, it is permanent and not associated with symptoms. The etiology is considered to be due to a degenerative process,[11] except for rare cases of alkaptonuric ochronosis.[8,10,13]

In childhood, the usual site of calcification is in the nucleus pulposus. It is frequently reversible and associated with localized symptoms of tenderness and paravertebral muscle spasm around the calcified area. The age distribution ranges from 5 months to 12 years. The symptoms disappear within a few days or weeks. However, the disappearance of the clinical manifestations does not always coincide with the resorption of calcium. The frequent correlation of the symptomatology with the roentgenological findings in the reported cases could not be considered coincidental; neither could it be accepted as a cause and effect. Twenty-five per cent are not associated with symptoms, and many symptomatic cases present no apparent radiological findings. To establish the consequential association of the clinical manifestations with the occurrence of calcification requires further investigation.

Etiologically, in children, the degenerative changes and alkaptonuric ochronosis can be eliminated, since in both conditions several years are required before calcific changes appear.[8,10,13] A metastatic infectious process is suggested by some authors whose patients had a moderate elevation of temperature and sedimentation rate and a leukocytosis.[1,5,10,15] Shorr and Adler[12] advocated a traumatic origin perhaps associated with hemorrhage in the nucleus pulposus and subsequent calcification.

Calcification of the intervertebral disk appears to be a self-limiting process and carries a good prognosis with simple conservative symptomatic measures. However, it should be emphasized that the finding of a calcified disk is not necessarily diagnostic; the patient should be observed for other possible causes of symptomatology, such as malignancy and granulomatous lesions.

In the first case presented in this report, in spite of elaborate diagnostic procedures, the diagnosis of calcified intervertebral disk was largely made by exclusion. The etiology was presumably traumatic; the calcification gradually diminished and was barely distinguishable by x-ray 10 weeks after the onset of symptoms. In the second case, in which a follow-up was not obtained, trauma also appeared to be the etiological factor.

Summary

Two additional cases of symptomatic calcified intervertebral disk in children are reported. Both cases were preceded by trauma; other etiological factors were reasonably eliminated in one case. The anatomy, physiology, and calcification of intervertebral disks are briefly reviewed.

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Subclinical Pneumocystis Pneumonitis

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In Europe, Pneumocystis carinii or interstitial plasma-cell pneumonia is frequent; it affects chiefly premature or otherwise debilitated infants, and it occurs sometimes in small epidemics.[1] The highest incidence of the infection is at the age of 4 weeks to 6 months. The mortality is high and is not influenced by therapy. The typical autopsy findings show an extensive pneumonia characterized by plasma-cell infiltration of the alveolar septa and a peculiar intra-alveolar material with many minute organisms. These have been identified as P. carinii and are generally accepted as the causative agent, although they have not been isolated in culture. Unequivocal transmission of the infection to experimental animals has not been accomplished. There is much controversy regarding the taxonomic position of the organism, which some consider a protozoon, others, a fungus.

In North America, Pneumocystic pneumonia is thought to be a rare disorder and thus far only a few isolated cases have been reported.[2-7] However, within one year after the first case of the infection had been recognized in this laboratory,[6] we have encountered the disease at autopsy as an incidental finding in two further patients, one of whom was a 10-year-old child. In these cases the pulmonary changes lacked many of the typical morphologic features of Pneumocystic pneumonia and consisted of a focal interstitial pneumonitis of bland appearance.

Report of Cases

CASE 1.—A 3-month-old white native American girl was found dead in her crib during the early morning of Jan. 10, 1958. After completion of the autopsy the following data were obtained from an interview with her parents. The infant weighed about 6 lb. at birth after normal gestation and delivery. She was breast-fed by the mother until the age of 6 weeks, when she weighed 8½ lb. At that time she was weaned and her diet was changed to baby foods and bottle feedings supplemented by a vitamin preparation which, however, was omitted some weeks before death. The child was a "good baby" and developed normally. The mother noted a yellow tinge of the skin five weeks after birth and a slight cold about four weeks before death. These manifestations subsided within a few days, and no medical advice was sought. On the day before death the infant appeared normal and healthy. She went to sleep as usual, at midnight took a bottle with good appetite, and went readily back to sleep. She failed to wake the parents at 4 a. m. when she was in the habit of requiring another feeding. At 5 a. m. the father awoke and found the infant dead.

The father, who was 24 years old and worked in a steel mill, enjoyed good health. The mother, who had had two previous pregnancies, was 23 years old, worked in a cotton mill, and was also healthy. The oldest child, a 3-year-old girl, was healthy. At the age of about 3 months she was treated by a physician for cough associated with low-grade fever. This illness lasted for about three weeks. The other infant, a girl, was born prematurely at 7-8 months of gestation and died 22 hours after delivery. No autopsy was performed.

The family lived in a small adequately furnished home located in a mill-town type section of a semi-rural community on the outskirts of Atlanta. The general cleanliness of the home was poor. The house was supplied with city water and had a septic tank. No pets were kept in the house, where mice, rats, and roaches were occasionally seen. Dogs and chickens were kept in the back yard. The family had had no known contact with anyone who had been in Europe.

Autopsy.—Gross Findings: The body was well developed and nourished, measuring 58 cm. in length and weighing 4,900 gm. The right lung weighed 53 gm. (normal 35 gm.); the left, 49 gm. (normal 30 gm.). The pleural surfaces were normal and transmitted a pinkish color with reddish-brown areas 3-5 mm. in diameter. The consistency

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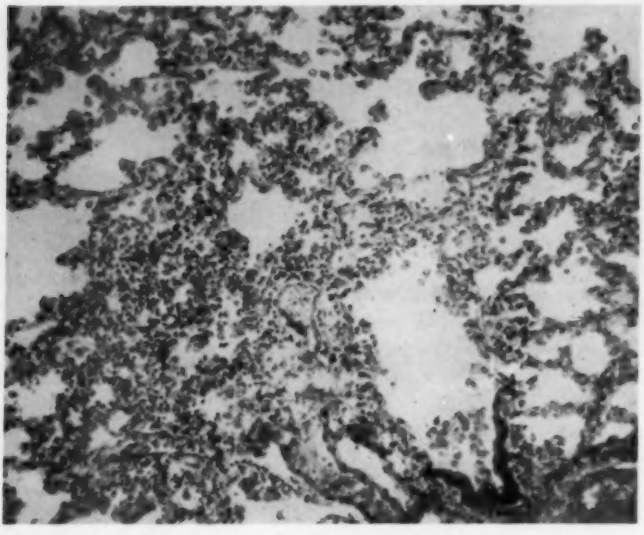

Fig. 1 (Case 1).—Slight focal interstitial pneumonitis. Hematoxylin and phloxine; × 125.

was slightly and uniformly increased. The cut surfaces revealed a similar color to that described above and showed no consolidation. All other organs and tissues including the brain were of normal size, weight, and appearance.

Histologic Findings: The lungs showed frequent minute foci located in close proximity to alveolar ducts (Fig. 1), where the septa displayed a slight cellular infiltration consisting chiefly of lymphocytes, occasional plasma cells, and large mononuclear cells (Fig. 2). In these areas the alveolar-lining cells were prominent and were shedding into the alveoli, where they were seen singly or as small aggregates of large mononuclear cells which extended into the alveolar ducts and terminal bronchioles. The mononuclear cells showed evidence of degeneration ranging from slightly sudanophilic intracytoplasmic vacuoles to necrosis. Spongy or honeycomb-like material, polymorphonuclear leukocytes, giant cells, fibrosis, hyaline membranes, and areas of septal wall necrosis were absent. Small numbers of Pneumocystis organisms, mostly cysts and, less commonly, free forms, were present among the alveolar contents. The organisms fre-

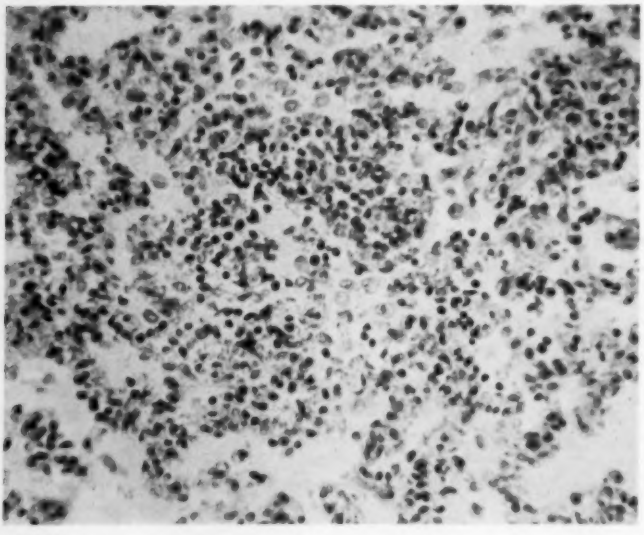

Fig. 2 (Case 1).—Interstitial pneumonitis with some large mononuclear cells in alveoli. Giemsa stain; × 265.

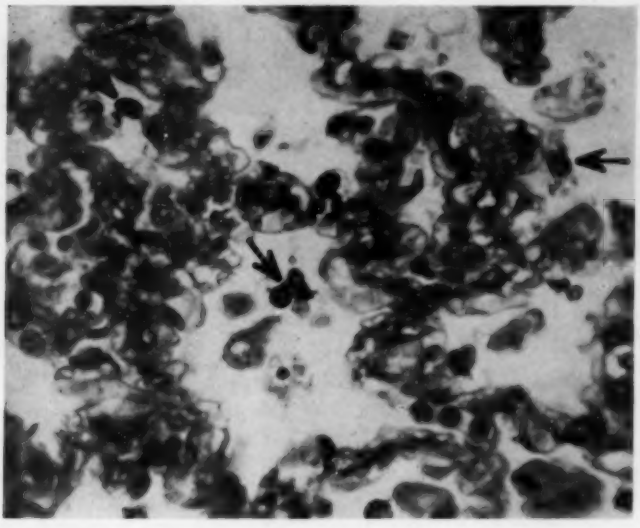

Fig. 3 (Case 1).—Occasional Pneumocystis cysts (arrows) in alveoli. Periodic acid-Schiff stain; × 672.

quently appeared to be in close contact with the alveolar-lining and intraluminal large mononuclear cells. They were not identified with certainty either within cells or in the septa. The organisms were readily demonstrated in sections with either Giemsa, Gomori's methenamine silver or periodic acid-Schiff (PAS) stains (Fig. 3) and in Giemsa-stained imprint smears of fresh lung tissue (Fig. 4 *A* and *B*). The smears also showed occasional Gram-positive cocci. Adjacent to the foci of pneumonitis were some atelectatic alveoli and larger areas of acute emphysema. There were no other findings except for depletion of the lymphoid tissues and acute congestion of the viscera. No cytomegalic inclusions were encountered, but the salivary glands were not examined.

Bacteriologic and Mycologic Findings: A culture of lung tissue yielded hemolytic Staphylococcus pyogenes var. aureus, coagulase-positive. No growth was obtained from heart blood. Tubes of Sabouraud's dextrose agar inoculated some weeks after autopsy with fresh-frozen lung tissue were incubated at room temperature as well as at 30 C and 37 C and yielded no fungus growth.

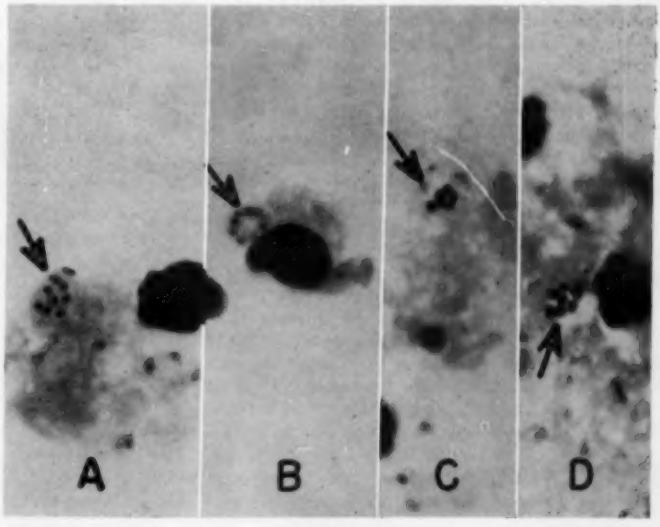

Fig. 4.—Pneumocystis organisms (arrows) in lung. *A* and *B* (Case 1), smears of fresh tissue, cysts with eight organisms; Giemsa stain; × 2,000 and × 1,800. Case 2, smears of formalin-fixed tissue. *C*, distorted cyst with six organisms. *D*, cyst with eight organisms. Giemsa stain; × 2,000.

CASE 2.—A 10-year-old white native American girl was admitted to Emory University Hospital on Jan. 19, 1958, with the diagnosis of glomerulonephritis and renal failure of about one month's duration. The past history revealed repeated bouts of tonsillitis. The last attack occurred 18 days before the onset of the present illness and subsided rapidly without medication. A routine physical examination had been performed in August, 1957, with negative results, and the parents had considered the child healthy and normal. The father, aged 49, enjoyed good health and worked as a tool-and-dye worker at an aircraft plant. The mother was 41 years old. She had been treated for pulmonary tuberculosis for one year in the state tuberculosis hospital but had been released three years previously and informed that the infection had been arrested. The patient had a 12-year-old brother and an 8-year-old sister. Both siblings were said to enjoy good health. The family lived in a small town 15 miles outside Atlanta. No other information was available concerning the patient's environment.

Physical examination revealed the following pertinent findings. The temperature was 98.6 F; pulse rate, 80 per minute; respirations, 16 per minute, and the blood pressure, 140 mm. Hg. systolic and 90 diastolic. The patient appeared ill and lethargic. There was moderate generalized edema. Funduscopic examination showed nothing significant. The respirations were slow and shallow. The heart and lungs were normal. The abdomen was protuberant, with some shifting dullness. Neurologic examination revealed nothing of note.

Urinanalysis showed a grossly bloody urine and 4+ albuminuria, specific gravity of 1.009, innumerable red blood cells, 4-6 white blood cells, 2-4 renal epithelial cells, and occasional hyaline and a few granular casts. The urine volume for 24 hours varied between 570 cc. and 1,220 cc. The urinary electrolyte determinations for 24-hour specimens ranged between the following values: sodium, 1.8 and 41.2 mEq. per liter; potassium, 15.4 and 35.5 mEq. per liter, and chlorides, 23.0 and 37.1 mEq. per liter. The blood hemoglobin was 7.2 gm. per 100 ml., with a terminal drop to 2.6 gm. per 100 ml. The total white blood cell count was 14,450 per cubic millimeter, with a differential count of 84% segmented cells, 10% lymphocytes, 3% monocytes, and 2% eosinophils. An L.E.-cell preparation was negative. The erythrocyte sedimentation rate was 104 mm. in one hour (Westergren). The total serum cholesterol was 214 mg. % on admission and fell later to 112 mg. %. The total plasma proteins were 3.9 gm. %, with 1.7 gm. % albumin and 2.2 gm. % globulin. Blood, serum, and plasma chemical determination ranged between the following values: NPN from 96 to 210 mg. %; CO_2 from 27.9 to 14.7 mEq. per liter; K from 5.2 to 4.4 mEq. per liter; Na from 107.0 to 148.5 mEq. per liter; Cl from 66.0 to 102.3 mEq. per liter; Ca from 7.4 to 8.8 mg. %, and P from 10.7 to 12.3 mg. %. Stool guaiac determinations varied from 1+ to 4+. A single urine culture obtained three weeks after admission yielded Escherichia coli.

The course in the hospital was characterized by increasing renal failure and progressive cardiac enlargement, with the blood pressure rising to 170 mm. Hg systolic and 110 mm. Hg diastolic. On the 16th hospital day an episode of slight convulsions of the right side appeared, and it recurred in a severer manner six days later. The lungs were repeatedly reported as clear on physical examination, but roentgenograms of the chest were not obtained. Among other medications the patient received 120 mg. of triamcinolone (Aristocort) from the 11th to the 13th hospital day and from then until the 54th day a total of 224 mg. of hydrocortisone derivative (Medrol). Various broad-spectrum antibiotics tetracycline ([Achromycin and Cosa-Tetracyn] and novobiocin [Albamycin]) were given from the 26th until the 72d hospital day. Terminally, the patient became comatose and died 112 days after admission.

Autopsy.—Gross Findings: The body was well developed but emaciated. The skin and mucous membranes were pale. The right lung weighed 180 gm.; the left, 240 gm. The pleural surfaces were normal. The consistency was normal, with some emphysema at the mediastinal margins and some 1.5-2 cm. areas of basal atelectasis. The cut surfaces exuded frothy pink fluid and were pale pink with reddish-brown areas of firmer consistency measuring 0.5-1 cm. in diameter, which were mainly located near the hilum. The bronchi contained a small amount of pinkish mucoid material. The heart weighed 215 gm. (normal 115 gm.) and showed hypertrophy of the left ventricle. The abdomen contained 100 cc. of clear straw-colored fluid. The lower esophagus revealed a superficial erosion measuring 2×1.5 cm. in diameter. Many minute superficial mucosal hemorrhages were seen throughout the small intestine. The pancreas weighed 20 gm. and was hard. The cut surfaces were not remarkable. The liver weighed 1,240 gm. and was congested. The combined weight of the adrenals was 9.5 gm., and on cut surfaces the cortices were pale yellow. The right kidney weighed 76 gm.; the left, 96 gm. The surfaces were finely and uniformly granular and pale brown in color, with many pinpoint-sized red spots. Demarcation of cortex and medulla was indistinct. The papillae on the left side were ulcerated and necrotic. The pelves and lower urinary tract were not remarkable. The brain weighed 1,220 gm. and showed cerebral edema. The left occipital lobe revealed a 2 cm. area of hemorrhage involving both gray and white

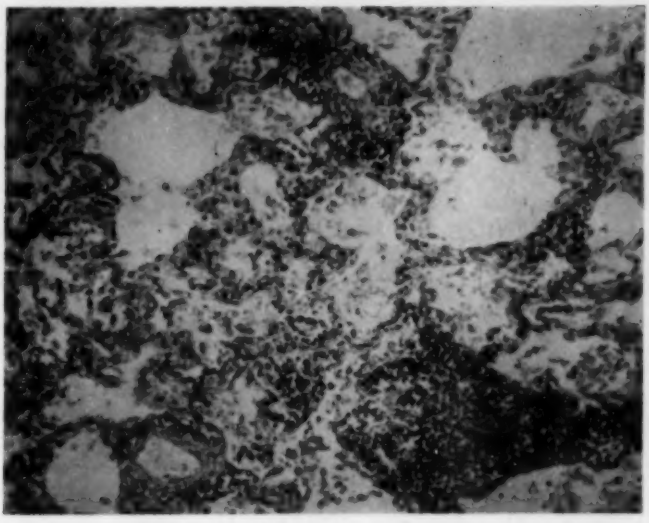

Fig. 5 (Case 2).—Foci of interstitial pneumonitis. Prominent alveolar lining cells and large mononuclear cells in alveolar lumens. Giemsa stain; × 125.

matter. Another hemorrhage, measuring 0.5 cm. in diameter, was present in the center of the pons.

Histologic Findings: Throughout the lungs, many small groups of alveoli in the immediate vicinity of alveolar ducts revealed a moderate infiltration of their septa by lymphocytes, occasional large mononuclear cells, and rare plasma cells (Fig. 5). In these areas the alveolar lining cells were prominent and tended to become detached into the alveoli, where they appeared as large mononuclear cells. These were present in moderate numbers, either singly or in small clusters, and frequently showed some sudanophilic vacuolation of the cytoplasm. Rare multinucleated giant cells were present in the alveoli (Fig. 6). Spongy material, polymorphonuclear leukocytes, hyaline membranes, and septal-wall necrosis were absent. Small numbers of Pneumocystis organisms, predominately cysts, were present in the alveoli. The organisms tended to form small clusters and generally appeared to be in close contact with the alveolar-lining and intraluminal-lining cells but were not seen either within cells or in the septa (Fig. 7). The Giemsa, Gomori's methenamine silver, and the PAS staining methods were employed for the demonstration of the agent in sections (Fig. 8). Many alveoli

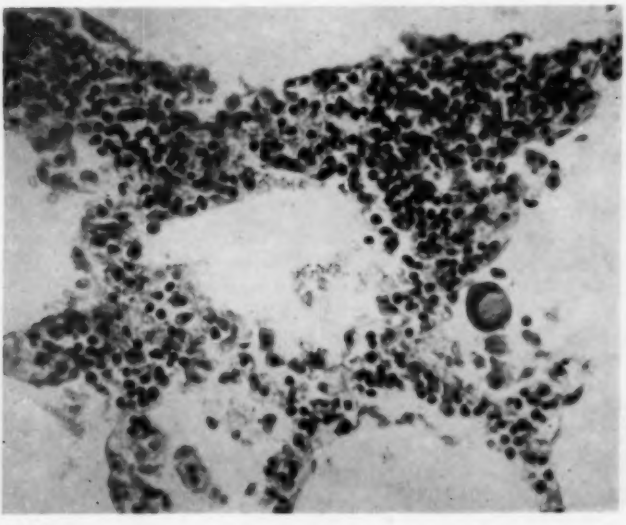

Fig. 6 (Case 2).—Interstitial pneumonitis. Multinucleated giant cell among alveolar contents. Giemsa stain; × 130.

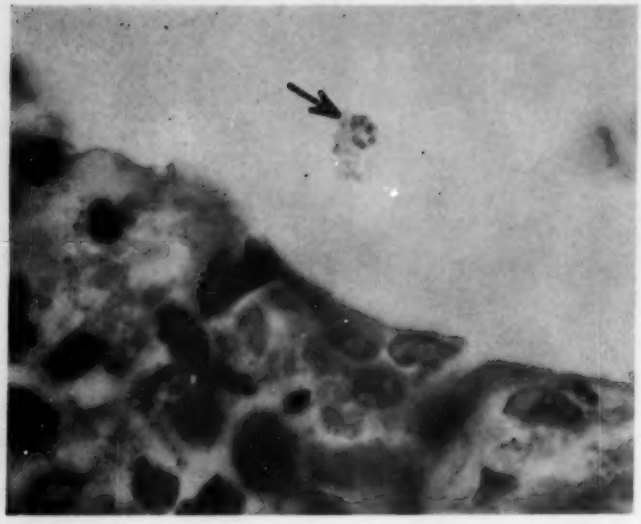

Fig. 7. (Case 2).—Pneumocystis cyst (arrow) in alveolar duct. Only five of the six organisms are visible. Giemsa stain; × 1,480.

between the areas of interstitial infiltration showed some emphysema, while others were partially collapsed. Throughout the lungs, some granular eosinophilic precipitate was present in the alveoli, a few of which also contained rare threads of fibrin. Some alveolar ducts and bronchioles contained masses of Gram-positive cocci and Gram-negative bacilli unattended by cellular response.

No imprint smears of the lung had been taken at autopsy, and no fresh-frozen material was available. An emulsion of formalin-fixed lung tissue in saline was prepared by grinding small fragments of tissue in a 40 gauge stainless-steel mesh sieve. Giemsa-stained smears of the emulsion revealed typical Pneumocystis cysts, as well as numerous cocci and bacilli. These preparations were less satisfactory than smears prepared from fresh or fresh-frozen tissue and did not permit the identification of single free Pneumocystis organisms. Furthermore, most Pneumocystis cysts were markedly distorted, but some were sufficiently preserved to reveal morphologic details and permit their identification (Fig. 4 *C* and *D*).

Additional histologic findings consisted of chronic glomerulonephritis; acute pyelonephritis with papillary necrosis of the left kidney; slight subacute

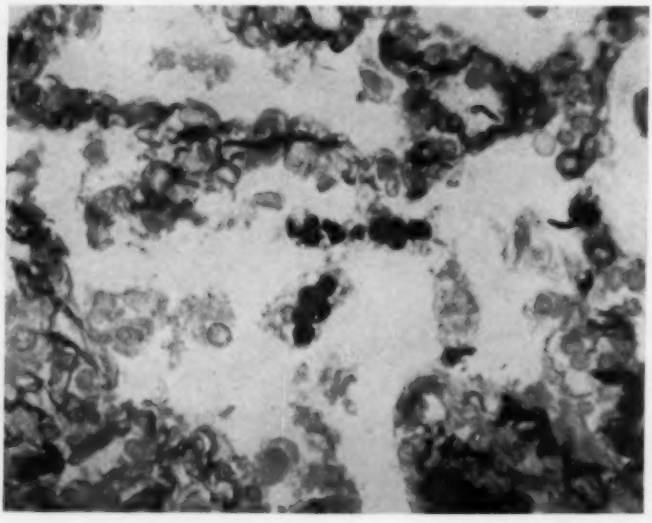

Fig. 8 (Case 2).—Clusters of Pneumocystis cysts (black) in alveolus. Gomori's methenamine silver-safranine; × 670.

focal pancreatitis and diffuse dilatation of pancreatic acini and ductules with inspissation of secretions; minimal interstitial myocarditis; subacute esophagitis; fatty metamorphosis of the liver; some hemosiderosis of spleen, liver, and bone marrow, and hyperplasia of bone marrow. There were old and recent thrombosis of an angiomatous malformation in the cortex of the left occipital lobe, with necrosis of the adjacent brain tissue and a small recent hemorrhage in the pons. No Pneumocystis organisms were identified outside the lung, and no cytomegalic or other inclusion bodies were found in any of the tissues. The salivary glands were not examined.

Cultures of lung tissue yielded hemolytic S. pyogenes var. aureus, coagulase-positive. No growth was obtained from the heart blood, and no fungus cultures were taken.

Comment

The incidental and bland character of the Pneumocystis infection in these two patients differs strikingly from the fatal and massive pneumonia generally described in this disease. The present observations suggest that latent and subclinical infection may actually be commoner than clinically manifest and progressing disease. It seems possible that the occurrence of the latter indicates a severe impairment of a factor in host-resistance, which appears to operate chiefly in early infancy.

In the two patients reported here the clinical data and gross postmortem findings did not, even in retrospect, suggest the diagnosis of Pneumocystis infection of the lung. Even histologically the Pneumocystis organisms were overlooked in the first patient in sections stained with hematoxylin and phloxine but were easily recognized with special stains after the diagnosis had been established in smears of fresh lung tissue. In the second patient, the organisms were demonstrated in sections without difficulty after the resemblance of the lung changes to those of the first case had been noted. In both patients the pulmonary lesions were focal and active but not extensive and showed no signs of rapid progression. The changes consisted of an infiltration of the alveolar septa by lymphocytes and by occasional plasma cells and large mononuclear cells and proliferation of alveolar-lining cells and some mononuclear cells in the alveoli and adjacent alveolar ducts and bronchioles. Pneumocystis organisms were present only in small numbers. This focal pneumonitis differed from Pneumocystis pneumonia as it has been described by most observers and as it appeared in the case of a 7-month-old boy previously reported from this laboratory.[6] The typical morphologic appearance of the disease as seen in Europe consists of a widespread pneumonia where the alveolar septa are infiltrated by chronic inflammatory cells, predominantly plasma cells, and where the alveoli are distended by a spongy or honeycomb-like material containing large numbers of organisms.[1] Some of the cases reported from North America, including the patient previously reported from this laboratory, show little or no plasma-cell infiltration of the alveolar septa,[3,6] although the alveoli are filled with spongy substance and organisms, while others reveal only a widespread and marked interstitial pneumonitis without demonstrable organisms.[5,9]

While in typical Pneumocystis pneumonia the massive consolidation of the lungs with subsequent pulmonary failure is the immediate cause of death, in the two cases reported here the Pneumocystis infection, which was obviously of some duration, did not produce an appreciable respiratory embarrassment and appears to be only an incidental finding. In the 10-year-old child the pneumonitis was mild and represented a complication which occurred in the course of chronic glomerulonephritis with progressive severe renal failure. A cerebral hemorrhage subsequent to thrombosis of a vascular malformation of the brain was the immediate cause of death. An agonal aspiration may account for the presence of staphylococci in the postmortem lung cultures. In the 3-month-old girl the Pneumoncystis pneumonitis was minimal and is regarded as an incidental finding. Death was attributed to an overwhelming staphylococcal infection possibly superimposed upon virus influenza, since at that time

several proven cases of Asian virus influenza with secondary staphylococcal invasion had occurred in this community. Unfortunately, no virus studies were carried out in this child. However, sudden death of infants has been attributed to Pneumocystis infection, which in a few cases was found at autopsy as a widespread pneumonia, while in others only small foci of interstitial pneumonitis were encountered.[10,11] In the latter, no postmortem bacteriologic studies are mentioned. Focal interstitial Pneumocystis pneumonia similar to that in the two current patients has been occasionally observed [12,13] and was thought to represent healing and abortive forms of the infection.[14] The morphologic appearance of the lesions reported here and the clinical status, as well as the laboratory data, in the second patient do not favor this interpretation.

It would appear that Pneumocystis pneumonia is not rare in North America, since three unrelated cases of the disease have been encountered within one year in this laboratory among only 53 autopsies of infants and children. Additional cases are being found by others either by review of old material [15] or in current patients.[7] Since interstitial pneumonitis is a common autopsy finding in infants, it will be of interest to determine how frequently this lesion is associated with the presence of Pneumocystis organisms.

The present findings and those reported in the literature suggest that in the pathogenesis of Pneumocystis infection a severe impairment of host resistance is essential before clinically manifest Pneumocystis pneumonia with respiratory distress and typical morphologic changes can develop. There is considerable evidence that latent infection is widespread in many different animals, but in the normal host the parasite seems to be avirulent and behaves like a saprophyte. In Europe and Brazil the parasite has been found in the lungs of many wild and domestic rodents, common laboratory animals, livestock, cats, and dogs.[1] In this laboratory smears of the lung from 13 normal rabbits were examined, and extremely rare but morphologically typical Pneumocystis organisms were demonstrated in 11 animals.[16] In the various animal species the agent produces no lesions, and generalized infection has been observed only in a single young dog.[17] Pneumocystis organisms have also been demonstrated without associated inflammatory reaction in the lungs of man. In the Netherlands Van der Meer and Brug [18] found the parasite in smears of the lungs in 3 of 104 patients examined at autopsy. In Germany Weisse and Wedler [19] demonstrated the agent at autopsy in smears and histologic sections of the lung in 23 of 152 unselected patients with a wide variety of diseases which did not include a single case of interstitial plasma-cell pneumonia. The age of the patients in whom the parasite was found ranged from 5 days to 79 years. These observations indicate that latent Pneumocystis infection is widespread among animals and probably is not rare in man.

All studies of Pneumocystis pneumonia emphasize that the disorder occurs chiefly between the ages of 4 weeks and 6 months and that it shows a definite predilection for premature or otherwise debilitated infants.[1] It is rare in older children [12,20] and has been recognized in only four adults.[21] Hamperl [21] has pointed out that the infection appears to have been present in two additional adults with cytomegalic inclusion pneumonitis reported by Wyatt et al.[22] All the adult patients were severely debilitated, and laboratory determinations revealed abnormalities such as reversal of the albumin-globulin ratio, anemia, and/or granulocytopenia or a reduced vitamin A blood level. One patient died of chronic myeloid leukemia, another, of Hodgkin's disease.

In infants Pneumocystis pneumonia is not generally associated with known alterations of plasma proteins, although its peak incidence corresponds with a transient physiologic decrease of the γ-globulin levels which begins one week after birth and reaches its lowest point at one month. At 3 months the level begins to rise, and adult values are reached at 2 years.[23] In a few

patients the disease appears to have been a complication of proven hypo- or agammaglobulinemia, and in these the infiltration of the alveolar septa by plasma cells was sparse or lacking.[5,7,21,25] The septal infiltrates in both patients described here contained only rare plasma cells, and the absence of these cells was noted in the lung of the patient previously reported from this laboratory.[6] Furthermore, a decrease in total plasma proteins with reversal of the albumin-globulin ratio was present in the 10-year-old child. It appears therefore that in the three patients with Pneumocystis infection of the lung observed in this laboratory a congenital or acquired deficiency of γ-globulins may have been present and may have been a predisposing factor for the occurrence of this infection. However, it is not probable that a deficiency of γ-globulin is a constant or even frequent occurrence in Pneumocystis infection, since the pulmonary lesions in the majority of patients are characterized by an infiltration with plasma cells from which, in fact, the name of interstitial plasma-cell pneumonia is derived. These cells are known to be closely related to the formation of γ-globulin and are markedly diminished in number or absent in hypo- or agammaglobulinemia.[26]

The available data in the literature indicate that spreading Pneumocystis infection occurs only when host resistance is significantly decreased.[1,12,25] Since agammaglobulinemia can be excluded in the majority of patients, the high incidence of the disease in early infancy suggest that there may exist at that time another defect of the body defenses consisting of an impaired mechanism which normally begins to appear one month after birth and contributes to the development of natural and/or acquired resistance during the first year of life. Thus the pulmonary lesions in Pneumocystis infection may be viewed as a reflection of the state of host resistance. Widespread pneumonia without plasma-cell infiltration would indicate virtual paralysis of γ-globulin formation. Typical extensive pneumonia with plasma-cell reaction may reflect severe impairment of body defenses not related to γ-globulin. Focal interstitial pneumonitis which eventually may be found to be the most frequent manifestation of the disease would represent a subclinical infection subsequent to some decrease in host resistance.

It is concluded that Pneumocystis infection of the lung is probably not rare in this country, that the disease may be subclinical, that it can manifest itself as a focal interstitial pneumonitis of bland appearance, and that it can occur in older children as well as in infants. Furthermore it is suggested that a severe impairment of host resistance must be present before clinically manifest Pneumocystis pneumonia develops.

Summary

Pneumocystis carinii infection of the lung was encountered as an incidental autopsy finding in two white native American girls, one 3 months old, the other 10 years old. The morphologic lesions consisted of a slight focal interstitial pneumonitis which had produced no detectable clinical manifestations.

These findings indicate that in this country pulmonary Pneumocystis infection may not be infrequent, particularly in subclinical form when it is associated only with mild and focal lung changes, and that it affects older children as well as infants. An appraisal of the available data suggests that in the normal host Pneumocystis carinii is of low virulence and that host resistance must be severely impaired before the parasite can proliferate sufficiently to produce clinically manifest disease.

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Addendum

Since the acceptance of this manuscript for publication two additional instances of subclinical Pneumocystis infection of the lung have been encountered in our laboratory. These occurred in Negro siblings. Historical, clinical, and epidemiologic data are not available.

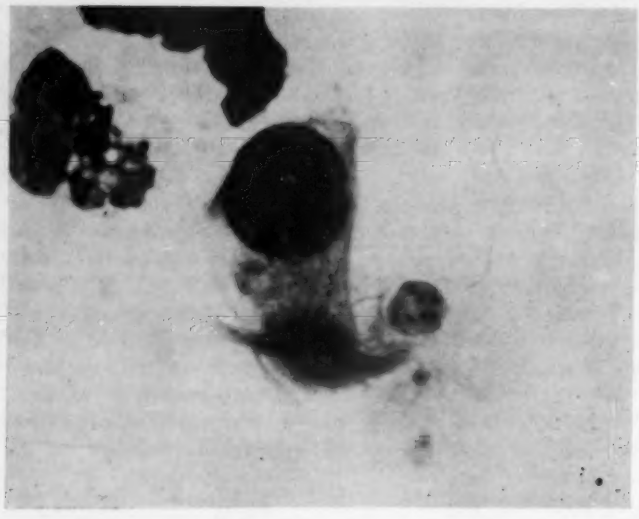

Fig. 9 (Case 3).—Lung smear. A pneumocystis cyst with eight organisms next to a ciliated bronchial epithelial cell. Giemsa stain; × 2,000.

CASE 3.—A 4-month-old Negro boy was found dead in his crib at 7:30 a. m. The child was seen apparently in good health at 12:30 a. m. of the same day, and there is no history of previous illness. A complete autopsy revealed a slight diffuse interstitial pneumonitis, some pulmonary edema, acute congestion of all viscera, and lymphoid depletion of the spleen, lymph nodes, and thymus. Postmortem cultures from the heart blood yielded a nonhemolytic Streptococcus, while a lung culture was overgrown with Proteus vulgaris. Sabouraud dextrose-agar cultures of lung tissue yielded no fungus growth. Giemsa-stained smears of fresh lung tissue revealed rare typical Pneumocystis cysts (Fig. 9). Histologic study of lung sections showed findings similar to those observed in Case 1, with occasional Pneumocystis cysts.

CASE 4.—During the work-up of the previous patient, it was noted that a complete autopsy had been performed two and one-half years previously on his 3½-month-old brother. This child was found dead at 1:30 p. m. The mother had fed the infant on the morning of his death and had noted nothing abnormal. The baby had been seen at weekly intervals by a public health nurse and appeared to be healthy. The postmortem findings consisted of a diffuse moderately severe interstitial pneumonitis, some pulmonary edema, acute congestion of viscera, and lymphoid depletion of spleen, lymph nodes, and thymus. A lung culture yielded Enterococcus, and no growth was obtained from the heart blood. Reexamination of the lung sections showed essentially similar findings as described above, with small numbers of Pneumocystis organisms, free forms as well as cysts.

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The Association of Various Factors and Hypernatremic Diarrheal Dehydration

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Although the number of patients admitted to the James Whitcomb Riley Hospital because of diarrheal dehydration has remained fairly constant during the past several years, the percentage of such patients who had hypernatremic (hypertonic) dehydration has increased steadily. The present study was undertaken to determine whether this increased incidence of hypernatremic dehydration was associated with factors in the therapy administered prior to referral to this hospital and if, as such, it reflected some changes in the manner in which the physicians of this state treated severe diarrhea. The four factors considered as possibly associated with hypernatremic dehydration were (1) the presence of preexisting brain damage; (2) the administration of parenteral fluids either intravenously or, as was usually the case, subcutaneously; (3) the use of noncommercial oral electrolyte solutions (O. E. S.), and (4) the use of a commercially available oral electrolyte preparation, Lytren (Mead Johnson & Company).

Case Material

The records of all children with hypernatremic diarrheal dehydration who were admitted to this hospital between Jan. 1, 1954, and Feb. 28, 1958, were examined. Patients without significant diarrhea were excluded from the study. Hypernatremia was defined as a serum-sodium concentration above 150 mEq. per liter. Each record was studied to determine if the patient showed evidence of brain damage before the onset of the diarrheal disease, if parenteral fluids had been given before admission to this hospital, or if the patient had received either Lytren or other solutions containing only glucose, salt, and water. Control data were obtained by examining, in an identical fashion, the records of all children admitted with isotonic diarrheal dehydration during the first three months of 1956 and 1957 and during January and February, 1958. No attempt was made to grade the severity of the preexisting brain damage or to determine the amount or composition of the parenteral fluids. The administration of Lytren and the other oral electrolyte solutions was considered to be of significance if the patient had received them for more than 12 hours. The total amount of the solutions that were given and the exact composition of the oral electrolyte solutions could not, in most instances, be determined from the records. The χ^2 test was used to determine if the frequency of these various factors differed significantly in the hypernatremic and control groups.

Results

The results of the survey are summarized in Table 1 and in the Figure. The total number of patients treated for diarrheal dehydration varied from a maximum of 202 in 1954 to a minimum of 153 in 1956. In January and February, 1958, 44 such patients were seen. The incidence of hypernatremic dehydration has increased markedly. Only 4% of the patients with diarrheal dehydration were hypernatremic in 1954. This figure increased to 6% in 1955, 10% in 1956, 16% in 1957, and 21% during the first two months of 1958.

In each year of the study some of the children with hypernatremic dehydration had, by history, preexisting brain damage. The percentage so involved varied from 37% in 1954 to 11% in 1955 and 1958. Of a total of 68 patients with hypernatremia 12 (17.7%) were brain-damaged before the onset of their diarrheal illness. In the control group of 156 children with isotonic dehydra-

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TABLE 1.—*The Association of Various Factors with the Development of Hypernatremic Diarrheal Dehydration*

Year	Total No. Patients	Hypernatremia	Control Group	Brain Damage		Parenteral Fluids		O. E. S.		Lytren		No Associated Factor	
				Hypernatremia	Control	Hypernatremia	Control	Hypernatremia	Control	Hypernatremia	Control	Hypernatremia	Control
1954	202	8	0	3	--	3	--	0	--	--	--	2	--
1955	158	9	0	1	--	5	--	0	--	--	--	3	--
1956	153	16	61	4	4	7	2	0	2	2	2	3	51
1957	164	26	60	3	4	6	2	3	2	7	4	7	48
1958	44	9	35	1	2	1	2	1	1	5	4	1	26
Total	721	68	156	12	10	22	6	4	5	14	10	16	125
	--	--	--	17.7%	6.4%	32.4%	3.8%	5.9%	3.2%	27.4 *	6.4%	23.5%	80.0%
				χ^2= 5.82		χ^2=34.5		χ^2= .512		χ^2=16.1		χ^2=60.1	
				P=<.02		*P*=<.001		*P*=<.5		*P*=<.001		*P*=<.001	

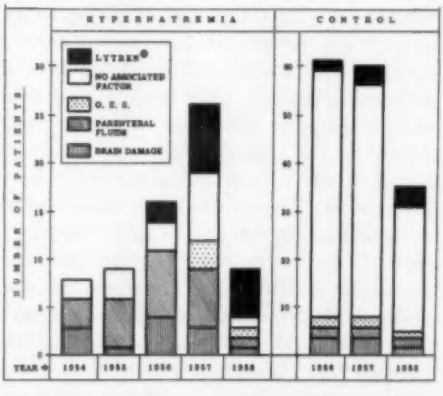
* Percentage of hypernatremic patients in 1956, 1957, and 1958.

tion only 10 (6.4%) had central nervous system pathology. This difference in incidence is statistically significant ($\chi^2=5.82$, $P=<.02$).

Nearly a third of the children with hypernatremic dehydration had received parenteral fluids either intravenously or subcutaneously before admission to this institution. Of the 68 patients with hypernatremia 22 (32%) had been so treated elsewhere. On the other hand, only 6 of 156 control subjects (4%) had received parenteral fluids. Again this difference is highly significant ($\chi^2=34.5$, $P=<.001$).

During 1954, 1955, and 1956 none of the children with hypernatremia had received a noncommercial solution composed of glucose, salt, and water. In 1957, three of twenty-six, and in 1958, one of nine children with hypernatremia received such a mixture. Thus, 4 of 68 were given oral electrolyte solutions. During this same period 5 of 156 children in the control group received similar oral solutions at home. This difference in incidence is not significant ($\chi^2=.512$, $P=<0.5$).

Lytren is a commercially available solution designed for oral administration to children with diarrhea. It is sold as a powder and, when properly reconstituted with water, supplies 380 Cal. from a carbohydrate base, 50 mEq. of Na, 20 mEq. of K, 4 mEq. of sulfate, 30 mEq. of chloride, 10 mEq. of phosphate, and 4 mEq. of lactate per liter. The manufacturers recommend that 2½ oz. of Lytren per pound of body weight be given in divided feedings each day. They also recommend that Lytren should not be given in place of parenteral fluids.[1] This preparation has become widely used in recent years. In 1956 only 2 of 16 children with hypernatremia and 2 of 61 children in the control group had received Lytren. In 1957 the figures rose to 7 of 26 and 4 of 60, respectively. In January and February, 1958, five of nine children with hypernatremia and four of thirty-five controls had been on Lytren before admission. During this interval, 14 of 51 children with hypernatremia



The relative frequency of the associated factors in children with hypernatremic diarrheal dehydration and in children with isotonic dehydration.

(27%) and 10 of 156 (6%) with isotonic diarrheal dehydration had received Lytren. This difference is highly significant statistically ($\chi^2=16.11$, $P=<.001$).

It is of interest that the history of children with hypernatremic dehydration disclosed that the large majority had either had previous brain damage or had received parenteral fluids, noncommercial oral electrolyte solution, or Lytren. One of these four factors was found to be associated with hypernatremia in 52 of 68 (77%) patients, whereas in the control group a similar history could be obtained in only 31 of 156 (20%) instances ($\chi^2=66.0$, $P=<.001$). The relative frequency of these factors in both the hypernatremic and control groups is illustrated in the Figure.

Comment

The increased incidence of hypertonic dehydration in children with preexisting central nervous system abnormalities has been previously reported.[2,3] The findings of the present study substantiate these earlier reports. However, the exact reason for this association is unknown. There was no predilection for a specific type of brain disease. Of the 12 hypernatremic infants who had central nervous system abnormalities, 2 had hydrocephalus; 2, microcephalus; 2, subdural hematomas; 1, severe retardation following meningitis, and 5, undifferentiated retardation.

The present investigators had expected to find a significant association of hypernatremia and the prior ingestion of oral electolyte solutions prepared by the patient's parents. These solutions were compounded, as a rule, from table salt, orange juice, sugar, and water. There is great danger that the parents will prepare this solution incorrectly and that the resultant mixture will contain more sodium than can be given safely. However, in this study no definite association between the use of such solutions and the subsequent development of hypernatremia could be demonstrated.

The statistical correlation between the use of parenteral fluids and the development of hypernatremia was striking. We have no knowledge as to the exact composition of the fluids given. However, in view of the hypernatremia that these fluids produced, it is a reasonable assumption that they often contained an isotonic concentration of electrolytes. The fluid losses, including those of the stool, are hypotonic in the child with diarrheal dehydration.[4,5] The administration of isotonic fluids will therefore produce hypertonicity.

The reason for the marked and significant association of hypernatremic dehydration and Lytren is not clear. Theoretically Lytren should be an ideal substance for use in the early management of diarrhea. The possibility exists that the Lytren was, in many cases, not prepared or administered in accordance with the manufacturer's recommendations. In our hands Lytren has been a satisfactory electrolyte preparation, as shown in the following study.

Forty children less than one year of age who had diarrhea were used as subjects. The majority were placed in the study upon the termination of intravenous fluid therapy. A few, whose illnesses were less severe, were placed in the study without prior intravenous therapy. Alternate infants, totaling 20 in each group, were given Lytren and a glucose-salt solution which contained 25 mEq. of Na, 25 mEq. of K, 25 mEq. of Cl, and 50 gm. of glucose per liter. Determinations were made of the serum sodium, potassium, chloride, CO_2 content, and nonprotein nitrogen at the time the infant was started on the study, at 24 hours, and at 48 hours. Both Lytren and glucose-salt were given ad lib. The volume taken was recorded as milliliters per 100 Cal. of caloric expenditure per day.[6] The results of this study are presented in Table 2. There are no significant differences between the two groups. The children on Lytren did not develop hypernatremia. However, at the time these children were placed in the study, their diarrhea had subsided and they did not have large extrarenal losses of hypotonic fluids.

TABLE 2.—*Comparison of Serum Electrolytes at Twenty-Four and Forty-Eight Hours of Infants on Lytren and "Glucose-Salt"*

		Onset					24 Hr.						48 Hr.					
Solution	Mean Age	Na, mEq/L.	K, mEq/L.	Cl, mEq/L.	CO_2, mEq/L.	NPN, Mg. %	Na, mEq/L.	K, mEq/L.	Cl, mEq/L.	CO_2, mEq/L.	NPN, Mg. %	Cc/100 Cal/Day	Na, mEq/L.	K, mEq/L.	Cl, mEq/L.	CO_2, mEq/L.	NPN, Mg. %	Cc/100 Cal/Day
Lytren	6 mo.	141	4.8	106	25.8	29	141	4.7	107	25.2	23	105	143	4.9	109	24.4	18	137
Glucose-salt	6 mo.	141	4.8	109	21.7	20	141	5.1	106	23.8	24	127	140	5.3	105	24.5	22	144

The fact that both parenteral fluid therapy and Lytren administration are associated to a significant degree with the development of hypernatremia cannot be interpreted as a blanket indictment of these forms of therapy. It does emphasize that care must be taken when using these substances. Parenteral fluids must be prepared properly. The use of subcutaneous fluids, particularly isotonic sodium chloride in the treatment of diarrheal dehydration is ill-advised. When parenteral alimentation is required, the intravenous administration of fluids is preferred. The electrolyte concentration of these fluids should be hypotonic.

Undoubtedly Lytren is useful in the treatment of diarrhea, particularly where dehydration has not occurred. However, in severe diarrhea Lytren must be used cautiously, and when parenteral fluids are needed Lytren must never be used in their stead.

Summary

Seven hundred twenty-one children with diarrhea were seen between Jan. 1, 1954, and Feb. 28, 1958. Of these , 68 had hypernatremia. The incidence of hypernatremia increased from 4% in 1954 to 21% in 1958.

Certain factors thought to be associated with the development of hypernatremia were studied. The findings of these studies are as follows.

Brain damage which preceded the acute illness was found in a significantly higher percentage of children with hypernatremia than in a control group with isotonic diarrheal dehydration.

The administration of parenteral fluids before admission to the James Whitcomb Riley Hospital was definitely associated with the occurence of hypernatremia.

The use of solutions compounded at home and containing glucose, electrolyte, and water could not be associated statistically with the occurence of hypernatremia.

The use of a commercial oral electrolyte solution, Lytren, in the treatment of diarrhea was significantly associated with the development of hypernatremia.

In a controlled study of children recovering from diarrheal dehydration, Lytren did not cause hypernatremia.

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Lysozyme Levels in Cystic Fibrosis of the Pancreas

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The significance of lysozyme in various body fluids has been discussed in several reviews.[1-3] In general, it is held that lysozyme, as it appears in tears and saliva, exerts a protective influence against bacterial invasion if present in an adequate concentration. Lysozyme exerts its protective effect by hydrolyzing certain structural polysaccharides in the bacterial cell wall, thus causing the dissolution of many bacteria, particularly cocci. Granulocytes[4] also contain "lysozyme-like" enzymes which are released when the cells are injured.[5-7] In areas where there has been a leukocytic proliferation due either to infection or irritation, relatively high levels of lysozyme may be observed.[4,8]

The study of the lysozyme activity of various body fluids from patients with cystic fibrosis was considered to be of interest in view of the almost constant development of chronic pulmonary suppuration in this disease. Abnormally high lysozyme levels were observed in the duodenal fluids of patients with cystic fibrosis when accompanied by pancreatic achylia. Elevated levels of lysozyme were also found in the gastric juice of patients with this disease. The effect of proteolytic enzymes in lowering the lysozyme levels was investigated in order to obtain some insight into the physiological relationship between these enzymes and the lysozyme content of body fluid.

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From the Division of Clinical Laboratories, the Department of Medicine, and The Children's Cancer Research Foundation, The Children's Medical Center, and the Department of Pediatrics, Harvard Medical School.

Methods and Materials

The method of Smolelis and Hartsell,[9] with some modifications, was used in the lysozyme determinations. "Bacto" lysozyme substrate, obtained from the Difco Laboratories,* was used. This substrate, consisting of lyophilized cells of Micrococcus lysodeiticus, was prepared by making a homogeneous suspension of the lyophilized organisms in M/10 phosphate buffer at pH 6.2. The suspension was diluted until a reading of 500 was obtained on the Klett Summerson photoelectric colorimeter with use of the 540 filter. Several crystalline egg lysozymes† were tested for use as standards and found suitable.

The material tested for lysozyme activity consisted of duodenal fluids, meconium, gastric juice, and saliva from patients with cystic fibrosis and from controls. The duodenal fluids tested had been stored in the Deepfreeze for varying periods of a week to several years. The fluids were gently thawed in lukewarm water, centrifuged for 10 minutes, and then filtered by suction with Johns Manville's celite analytical filter aid and diluted with phosphate buffer.

Normal meconium, as well as three samples of meconium from meconium ileus cases, was also tested for lysozyme. Wherever possible, fresh specimens of meconium were obtained and kept in air-tight jars in a Deepfreeze to avoid evaporation. Aqueous extracts were prepared by mixing weighed amounts of meconium with two to eight parts of water, depending upon the consistency and water miscibility of the meconium. The extracts were allowed to stand for one hour at room temperature, after which they were centrifuged and clarified by suction as described above.

Gastric juice was obtained from healthy and sick persons and from patients with cystic fibrosis of the pancreas. Residuum, as well as histamine-stimulated gastric juice, was obtained. After clari-

* Difco Laboratories, Detroit 1.

† Obtained from the Worthington Biochemical Sales Company, Freehold, N. J., Difco Laboratories, Detroit 1, Armour Laboratories, Chicago, and several recrystallized lysozymes from Prof. Joseph Smolens, University of Pennsylvania, School of Medicine, and the Philadelphia Children's Hospital.

Lysozyme in Duodenal Fluid

	Control Group					Patients with Cystic Fibrosis		
	Healthy and Sick Children	Celiac Syndrome	Parents of Children with Cystic Fibrosis	Well Siblings of Cystic Fibrosis	Total	Complete Pancreatic Insufficiency	Partial Pancreatic Insufficiency	Total
Patients, no.	41	6	4	11	62	29	4	33
Lysozyme absent, no.	37	5	4	7	53	2	2	4
Lysozyme present, no.	4	1	0	4	9	27	2	29
Lysozyme present, %	(9.8%)	(16.7%)		(36.4%)	(14.5%)	(93.0%)	(50%)	(88%)
Range of activity, μg/ml.	0-51	15	0	0-18	0-51	0-292	0-30	
Mean	4.0	2.5	0	3.8	3.5	99.5	13.3	

fication, as previously described, the juice was neutralized with solid $NaHCO_3$ or 0.2M $NaHCO_3$ until a pH of about 6.2 was obtained. Dilutions of this material with phosphate buffer were made as required.

Mixed saliva was obtained from healthy and from sick children and from other donors. It was clarified and diluted with phosphate buffer.

Results

Duodenal Fluids.—Duodenal fluids from two groups were tested for lysozyme. One was a control group and the other a group of patients with cystic fibrosis showing either complete pancreatic insufficiency or only partial or no pancreatic insufficiency.[10,11] These results are shown in the Table. It will be observed that most of the patients in the group consisting of healthy and sick children showed an absence of lysozyme; only 4 out of 41 patients had any demonstrable enzyme, with an average of 3.5μg. per milliliter for the control group. In contrast, 29 patients with cystic fibrosis, having complete pancreatic insufficiency, averaged 99.5μg. per milliliter. Four patients having partial pancreatic insufficiency had a mean value of 13.3μg. per milliliter.

Digestion Experiments with Duodenal Fluid.—Since normal duodenal fluid contains either little or no lysozyme, it was considered desirable to investigate some of the factors which might be responsible for the elevated lysozyme content of the group of patients with cystic fibrosis.

In cystic fibrosis with pancreatic insufficiency, enzymes are either low or absent in the duodenal fluid.[10,11] Since lysozyme is a protein or polypeptide with a molecular weight of about 25,000,[3] it might conceivably be digested by proteolytic enzymes. Normal duodenal fluid (containing proteolytic enzymes) was mixed with samples of duodenal fluid from patients with cystic fibrosis (containing lysozyme but no proteolytic enzymes) and incubated at 37 C. It was observed that normal duodenal fluid destroyed the lysozyme activity within one hour. Controls, where phosphate buffer pH 6.2 was substituted for normal duodenal fluid and incubated under similar conditions, were unchanged. Pancreatin ‡ also destroyed lysozyme at a slower rate.

The samples of duodenal fluid presented in the Table were tested for proteolytic and other enzymes. In the group of patients having cystic fibrosis of the pancreas with complete pancreatic insufficiency, the lysozyme values averaged 99.5μg. per milliliter. There were 2 specimens with no lysozyme and 12 specimens with lysozyme levels from 13μg. to 52μg. per milliliter. The balance (15 specimens) ranged from 80μg. to 292μg. per milliliter. The control group of healthy and sick children had 4 specimens with lysozyme values from 26μg. to 51μg. per milliliter, 37 specimens in this group having no lysozyme. A comparison of the proteolytic enzyme levels in both groups showed that in the control group the proteolytic enzymes were in the normal range, while in the group of patients with cystic fibrosis, regardless of the lysozyme levels,

‡ Pancreatin, U. S. P. Parke, Davis & Company, Detroit.

the proteolytic enzymes were either abnormally low or absent.

Meconia.—Ten normal meconia and three meconia obtained in surgical specimens from patients with meconium ileus [12,13] were tested for lysozyme. The 10 normal meconia had no lysozyme. Three meconia from patients with meconium ileus gave values of 101μg., 44μg., and 45μg. of lysozyme per gram of meconium. All three specimens showed the presence of large amounts of protein.[13]

Gastric Juice.—Gastric juice was tested from five patients with cystic fibrosis and 13 controls where the disease was excluded on laboratory and clinical grounds. The control group consisted of nine adults and three children, aged 2, 2½, and 5 years. Specimens were obtained before and after histamine stimulation. In several cases, residuum after overnight fasting was also collected. There was considerable variation in the lysozyme content of the controls, ranging from 0μg. to 299μg. per milliliter and averaging 65μg. per milliliter. The lysozyme in gastric juice from patients with cystic fibrosis ranged from 63μg. to 286μg. per milliliter, averaging 159μg. per milliliter.

In the control group, there were two patients with chronic bronchitis with lysozyme values of 153μg. and 299μg. per milliliter. In the remaining patients having no respiratory symptoms, the maximum value was 82μg. per milliliter. The average lysozyme level of the control group becomes 40μg. per milliliter if we exclude the two patients with bronchiectasis.

Digestion Experiments with Gastric Juice. Using the same technique employed in the experiments with duodenal fluid, mixtures of gastric juice containing lysozyme and undiluted gastric juice without lysozyme were tested. Normal gastric juice in each instance completely destroyed the lysozyme present within four hours. There was little or no destruction of lysozyme in controls where 0.01 N HCl was substituted for normal gastric juice and incubated under the same conditions.

Several specimens of gastric juice containing lysozyme were allowed to incubate to determine whether or not autodigestion would occur. In 8 out of 10 specimens complete autodigestion occurred during 24 hours of incubation. In two specimens where little autodigestion occurred, the addition of crystalline pepsin § was without effect.

In an attempt to explain this phenomenon, pepsin determinations were made on gastric juice from the controls as well as from the cases of cystic fibrosis. Pepsin was determined by the method of Bucher, Grossman, and Ivy.[14] In general, it was observed that there was no significant difference between the pepsin levels of the controls and of the cases of cystic fibrosis.

Cabezas and Rubina [15] found that gastric juice did not inactivate lysozyme. Differences in the method of assay, as well as other conditions of these experiments (period of incubation), may explain the discrepancy between our experiments and theirs.

Saliva.—Lysozyme levels were determined in saliva from patients with cystic fibrosis and from controls. The control group consisted of five adults and eight children ranging in age from 6 to 12½ years. The adults had a higher level of lysozyme than the children. The average levels were 143 and 71 in the control group for the adults and children, respectively; the over-all average was 104μg. The range of the adult controls was from 82μg. to 230μg. and the children, 16μg. to 99μg. per milliliter. Saliva from 17 patients with cystic fibrosis was examined. The ages of the patients varied from 3 to 15 years, averaging 7.7 years. The average lysozyme level was 95μg., and the range was from 10μg. to 221μg. per milliliter.

Digestion Experiments with Saliva.—The effect of gastric juice and crystalline pepsin on lysozyme from saliva, as well as crystalline egg lysozyme, was investigated in a manner similar to that already described for gastric juice.

§ Worthington Biochemical Sales Company, Freehold, N. J.

It was observed that undiluted normal gastric juice (lysozyme-free) at the end of four hours had completely digested the salivary lysozyme. Similar dilutions of crystalline egg lysozyme were also completely inactivated at the end of four hours. The control dilutions, consisting of saliva diluted with 0.01 N HCl, showed very little change in lysozyme level during the incubation period. It was also observed that crystalline pepsin inactivated both crystalline and salivary lysozyme.

Comment

The observed lysozyme levels in duodenal fluid show that elevated values are found when there is a deficiency of pancreatic enzymes. It is conceivable that if a larger sample of duodenal fluids from healthy and sick children were analyzed, temporarily elevated values might be observed, especially in patients with bronchiectasis [1] or intestinal irritative or inflammatory processes.[4,5] Normal duodenal fluid and gastric juice contain enzymes which inactivate lysozyme obtained from a variety of sources. The equivocal results obtained in the auto-digestion experiments, with and without pepsin, indicate that there may be factors in gastric juice that inhibit the autodigestion of lysozyme. Normal levels of acid and pepsin in gastric juice from patients with cystic fibrosis rule out these factors as contributing causes.

The lysozyme of gastric juice is derived from the gastric mucosa, as well as from swallowed sputum and saliva. It will be recalled that in the control series of gastric juices, the highest lysozyme levels were obtained from patients with bronchitis. In a similar fashion, pulmonary infections associated with cystic fibrosis may be responsible for an increase in the gastric lysozyme level in these patients. Salivary lysozyme, however, is apparently in the normal range in patients with cystic fibrosis.

The experiments reported in this paper do not permit any precise localization of the source of lysozyme in the duodenal fluids. Under normal physiological conditions, undigested lysozyme from the stomach may reach the duodenum, particularly if the gastric lysozyme level is high. Under the conditions employed in the direct collection of duodenal fluid, however, gastric juice is practically excluded from the specimen. Accordingly, the observed lysozyme may arise from any of the other related systems that contribute to the duodenal juice. In the postmortem study of patients with cystic fibrosis, the stomach and duodenum are, in most instances, free of evidence of inflammation.‖ Occasionally, inflammation of extrahepatic bile ducts may be seen. In a majority of the cases a very mild chronic inflammation of the pancreas is found. Since pancreatic enzymes destroy lysozyme, the absence of lysozyme in duodenal juice from presumably healthy subjects does not rule out the possibility of a normal secretion of this enzyme in healthy subjects.

Summary

The object of this study was to investigate the lysozyme levels of various body fluids from patients with cystic fibrosis of the pancreas and from appropriate controls and to explain the differences observed. Determination in duodenal fluid from patients (cystic fibrosis) showed an elevated level averaging 99.5μg. per milliliter. In a control series of duodenal fluids, zero lysozyme levels were generally observed. In a few control cases low levels were found, the over-all average being 3.5μg. per milliliter. Normal duodenal fluid rapidly digested lysozyme found in duodenal fluids of patients with cystic fibrosis. Gastric juice lysozyme levels from patients were higher than from the control group, the average being 159μg. and 65μg. per milliliter, respectively.

These investigations suggest that the determination of the activity of lysozyme in gastric or duodenal fluid cannot be used as a diagnostic test for cystic fibrosis.

‖ Personal communication from Dr. John M. Craig, Pathologist, The Children's Medical Center, Boston, Massachusetts.

Normal gastric juice (lysozyme-free) digested lysozyme from abnormal gastric juice containing lysozyme. The pepsin and pH levels of gastric juice from patients with cystic fibrosis were essentially in the normal range. Salivary lysozyme levels from patients with cystic fibrosis and from the controls were essentially in the same range. Crystalline pepsin and normal gastric juice digested both salivary and crystalline egg lysozyme. Normal meconium is free from lysozyme, while meconium from surgical specimens obtained from patients with meconium ileus contained appreciable amounts of lysozyme. Various explanations are advanced to clarify the reasons for the abnormal lysozyme levels observed in cystic fibrosis.

Children's Hospital, 300 Longwood Ave. (15) (Dr. Shwachman).

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Vasopressin-Resistant Diabetes Insipidus

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In 1947 Williams and Henry [1] introduced the term "nephrogenic" to describe a form of diabetes insipidus in patients whose symptoms were not relieved by the administration of vasopressin. The disease had been mentioned in 1923 by Veil,[2] who noted the same lack of response in some of his patients. The published literature until 1945 describing this type of diabetes insipidus (as well as the ordinary type in which vasopressin is effective) was reviewed by Forssman,[3] who also included eight cases of his own. The supposition that the kidney was at fault was made by Williams and Henry [1] on the basis that renal function was found to be somewhat impaired in the patient whom they studied. They found the renal plasma flow (R. P. F.), the maximal tubular excretion (Tm) of iodopyracet (Diodrast), and the urea clearance to be low, in addition to subnormal renal concentrating powers after a period of water deprivation or after administration of vasopressin.

Since 1945 about 120 other patients have been described, about 50 of which were observed by the authors.[4-21] * The remainder were other members of the families of the patients seen. According to these reports, the disease, which usually evidences itself soon after birth, is often familial and occurs more frequently in males. A single recessive sex-linked gene has been implicated,[1,18,19,21] but there is little doubt that females are frequently affected [18,20,21] and that a dominant gene is involved. There are also instances of isolated cases where evidence of disease in other members of the family was not or could not be obtained.[5-8] During episodes in which there is insufficient water intake or some other aggravating factor, there may occur elevations in concentration of sodium, chloride, and urea in the serum which return to normal when the patient drinks sufficient quantities of water. Assays of antidiuretic activity in the plasma and urine of the patients have been reported to show considerable activity.[5,10]

Occasionally subnormal values for renal-function tests have been reported when studies were made even after the patients had drunk sufficient water and were experiencing relative remission of symptoms,[1] but generally renal function, aside from failure to concentrate the urine in the face of adequate water deprivation, has been reported as normal.[1,10] Measurements of rates of renal excretion of 17-ketosteroids have revealed that these are normal, as have been other measurements of adrenal function.[10]

It is the purpose of this report to describe eight new patients with this disease in three generations of a family. All except one were seen by us, and some were studied fairly extensively. In addition to adding eight new patients to the list already published, this family is of interest because it sheds some light on the mode of inheritance of this disease; because it demonstrates that whereas some members may be severely affected, others seem to get along reasonably well without any need of medical assistance, and because additional studies of renal and adrenal function have uncovered certain facts not heretofore published about this disease.

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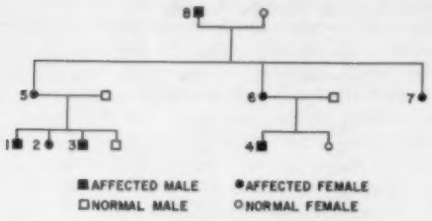
Technical assistance was given by Miss S. Graziano, Miss D. Wetherall, and Mr. J. Woods.

* The exact number is difficult to assess because the same patient is often reported on more than one occasion by the same or another author.



Case Histories

The family pedigree of our patients may be seen in the Figure. The numbers next to the symbols are the case numbers used below. The patients are all Negroes.

CASE 1.—Patient 1 was considered a normal full-term infant at birth when his weight was 3,000 gm. However, at 2 weeks of age medical advice was sought because he was noted to be listless and feverish. Routine urinalyses at this time and on all subsequent occasions were normal. The cause of his fever remained obscure, and at the age of 3 months, having failed to grow normally, he was admitted to the Kings County Hospital. His weight at this time was 4,200 gm. He was noted always to be excessively thirsty and to urinate excessively.

At this time, the concentration of his serum sodium was found to range from 154-176 mEq. per liter; chloride, 114-125 mEq. per liter, and carbon dioxide content, 12-24 mEq. per liter (Table 1). His thirst and polyuria failed to respond to 0.4 units of vasopressin administered intramuscularly. In one period of 24 hr., 454.6 mg. amino nitrogen (1,569 mg. per square meter) was excreted, the normal rate being less than 500 mg. per square meter per 24 hr. Intravenous and retrograde pyelography revealed no structural anomalies. Aldosterone excretion in the urine was normal in quantity. He was discharged from the hospital after these studies, but most of his life until the age of 16 months was spent in one hospital or another. At this age he was readmitted to the Kings County Hospital for further study.

TABLE 1.—*Serum Analyses in Vasopressin-Resistant Diabetes Insipidus*

Case No.	HCO_3, mEq/L.	Na, mEq/L.	K, mEq/L.	Cl, mEq/L.	pH	Urea Nitrogen, Mg/100 Ml.
1	22	176	5.0	125	7.44	34
1*	12	184	4.4	139	7.26	50
1	21	144	4.0	106	7.33	11
2	21	155	4.0	100		10
3	22	144	4.5	99	7.42	14
4	28	155	3.7	100	7.33	11
5	25	149	3.8	103	7.45	14
6	27	148	4.5	103	7.46	11
7	25	147	4.0	100	7.31	10

* After administration of 1.35 gm. ammonium chloride over an 18-hour period.

Fever, fretfulness, growth retardation, polyuria, and polydipsia were the main complaints at this time. His weight was now only 5,800 gm., and his length was 61.0 cm. His capacity for ingesting water was great, and it was remarkable to watch this small infant gulp down 8 to 12 oz. of water at a time with great dispatch.

After 10 hours of water deprivation (it was not considered prudent to prolong this period) the solute concentration in his urine was 630 mOsm. per liter (the normal value being over 1,000 mOsm. per liter). His powers of base conservation were then tested, and the plan was to administer 6 gm. ammonium chloride per square meter daily for four days. After only one day the carbon dioxide content of the serum fell to 12 mEq. per liter and the serum pH, to 7.26. He was noted to be breathing heavily. Administration of the drug was therefore discontinued. His urea clearance was 12.7 ml. per minute per square meter.

At the age of 21 months he was readmitted to the Kings County Hospital suffering from fever and cough. Type 14 pneumococcus was isolated from a blood culture. After the subsidence of this infection, his inulin clearance was found to be 74.3 ml. per minute per square meter (normal, 60-80) and his urea clearance was 69.7 ml. per minute per square meter (normal, 45-75) (Table 2). This time when ammonium chloride was administered for three days metabolic acidosis did not develop and the excretion of ammonia was found to be 72.2 mEq. per 24 hours per square meter (normal, more than 40) and the titratable acidity, 23.6 mEq. per 24 hours per square meter (normal, more than 20). He excreted 239 mg. amino nitrogen per 24 hours per square meter (Table 2).

At the time of writing his weight has increased to 8.63 kg. and his height, to 72 cm. He appears to be growing and developing at a much more satisfactory rate in the second half of his second year of life than he was previously, but he continues to ingest about 3 liters of water daily (7.5 liters per square meter). At age 2 years, he is now beginning to walk and verbalize and, although mental development has begun to accelerate in the past six months, his psychomotor status is still considerably retarded.

CASE 2.—Patient 2 is a 4½-year-old girl weighing 18.0 kg., whose length is 111.1 cm. She has suffered from polyuria and polydipsia since shortly after birth. She appears to have normal mental development. After 12 hours' water deprivation the concentration of total solutes in her urine was 425 mOsm. per liter. After receiving 6 gm. am-

TABLE 2.—*Renal Function Studies in Vasopressin-Resistant Diabetes Insipidus*

Case No.	Total * Solutes, mOsm/L.	Inulin Clearance, Ml/Min/m.²	Urea Clearance, Ml/Min/m.²	NH_4 Production, mEq/Day/m.²†	Titrable Acidity mEq/Day/m.²†	Amino Nitrogen, Mg/Day/m.²
1‡	613		18.0	46.5	22.3	1,569
1§	578	74.3	60.7	72.2	23.6	230
2	425			120.0	31.9	491
3	722		65.0	76.3	47.7	782
4	551	73.2	46.7	77.1	34.0	1,471
5	455					
6	448					
7	163					148

* After 12 hr. of water deprivation.
† After administration of ammonium chloride (6 gm. per square meter daily for three days).
‡ Patient was severely ill.
§ Patient was in remission.

monium chloride per square meter for three days, her ammonia production was 120 mEq. per 24 hours per square meter and titratable acidity, 31.9 mEq. per 24 hours per square meter (Table 2). The concentration in her serum of several substances was measured, namely: sodium, 155 mEq. per liter; potassium, 4.5 mEq. per liter; chloride, 100 mEq. per liter; bicarbonate, 21 mEq. per liter and urea nitrogen, 10 mg/100 ml. (Table 1).

CASE 3.—Patient 3 is a 2½-year-old boy weighing 15.9 kg., whose height is 104.1 cm. He too has suffered from polyuria and polydipsia since shortly after birth. After 12 hours of water deprivation, the osmolarity of his urine was found to be only 722 mOsm. per liter. The concentration in his serum of sodium was 144 mEq. per liter; potassium, 4.5 mEq. per liter; bicarbonate, 22 mEq. per liter; chloride, 99 mEq. per liter, and urea nitrogen, 14 mg/100 ml. The pH of his serum was 7.42. After ingesting 6 gm. of ammonium chloride per square meter for three days, he excreted 76.3 mEq. ammonia and 47.7 mEq. titratable acid per 24 hours per square meter. His urine contained 782 mg. amino nitrogen per square meter per 24 hours. This child has normal mental development.

The fourth child in this family is apparently normal. After 12 hours of water deprivation the total solute concentration in the urine was 1,120 mOsm. per liter. His inulin clearance was 122 ml. per minute per square meter and his urea clearance, 45.2 ml. per minute per square meter. After ammonium chloride ingestion (6 gm. per square meter for three days), he excreted 120 mEq. ammonia and 25.5 mEq. titratable acid per 24 hours per square meter.

CASE 4.—Patient 4 is a 4½-year-old boy, weighing 14.1 kg. and measuring 104 cm. in length. His daily intake of fluid exceeds 3 liters per square meter. His mental development appears to be normal. After 12 hours of water deprivation the total solute concentration in his urine was found to be 557 mOsm. per liter. The following renal function studies were also done: inulin clearance, 73.2 ml. per minute per square meter; urea clearance, 46.7 ml. per minute per square meter; ammonia production, 77.1 mEq. per 24 hours per square meter, and titratable acidity, 30.4 mEq. per 24 hours per square meter (the last two after ingesting 6 gm. ammonium chloride per square meter for three days). The following analyses were made on his serum: sodium, 155 mEq. per liter; chloride, 109 mEq. per liter; potassium, 3.7 mEq. per liter; bicarbonate, 28 mM. per liter; pH, 7.33, and urea nitrogen, 11 mg/100 ml. This boy has never been known to have unexplained high fever or dehydration. His brother is apparently perfectly normal and does not have polyuria or polydipsia.

CASE 5.—Patient 5 has suffered from polyuria and polydipsia since birth. She has grown and developed normally, however (height, 170 cm.; weight, 64.1 kg.). The concentrations of the following substances were measured in her serum: sodium, 149 mEq. per liter; potassium, 3.8 mEq. per liter; chloride, 103 mEq. per liter; urea nitrogen, 14 mg/100 ml. The pH of her serum was 7.45. After 14 hours of water deprivation the concentration of total solutes in her urine was found to be 455 mOsm. per liter.

CASE 6.—Patient 6 is the mother of the child designated as Patient 4. She is 24 years old and weighs 137 lb., and her height is 170 cm. She has had polyuria and polydipsia all her life but has been quite normal in every other respect. After 12 hours of water deprivation the concentration of total solutes in her urine was 440 mOsm. per liter. In her serum the following concentrations were found: sodium, 148 mEq. per liter; potassium, 4.5 mEq. per liter; chloride, 103 mEq. per liter; urea nitrogen, 11 mg/100 ml., and pH, 7.46.

CASE 7.—Patient 7 is the sister of the two women designated as Patients 5 and 6. Her height is 174 cm. and her weight, 60.4 kg. She has suffered from polyuria and polydipsia since birth but is apparently normal otherwise. A urine concentration test was attempted, and the specimen of urine obtained had a concentration of total solutes of only 143 mOsm. per liter. It is doubtful that this specimen was obtained after complete abstention from water. The concentration of the following substances were found in her plasma: sodium, 147 mEq. per liter; potassium, 4.0 mEq. per liter; chloride, 100 mEq. per liter; urea nitrogen, 10 mg/100 ml., and pH, 7.31. Routine chemical and microscopic analyses of the urine of this subject as well as that of all the others mentioned above were always normal.

Comment

The most remarkable feature of the disease as manifested by these patients is its extraordinary variability. One patient (Patient 1) suffered not only from intense polyuria and thirst but during the first few months of his life he grew poorly, was retarded in psychomotor development, and had recurrent episodes of fever and dehydration during which he became critically ill and had to be admitted to the hospital in an emergency. On the other hand, all the other members of the family have suffered only to a mild extent, by comparison, and, aside from the inconvenience and discomfort associated with polyuria and polydipsia, have been able to lead normal lives. Three of the adults with the disease have married and have children, while three of the affected children attend school, have grown normally, and do not appear to be seriously inconvenienced by their inability to get along with a normal allowance of water.

The fact that the disease occurs in both males and females is worthy of note. It has been considered that this disease occurs in the female in the "carrier state" only and that symptoms occur only in the male. If our series of patients is representative of all of those who have the disease, then it appears likely that the disease occurs in both sexes. In this connection it should be noted that the term "carrier state" has been used in referring to female subjects who suffered from polyuria and polydipsia.[18,21] It appears to us that this is a contradiction in terms and that any subject with symptoms ought not to be referred to as a carrier. Three of the male and four of the female members of the family reported here had only polyuria and polydipsia. To refer to the females as carriers and to the males as patients with the disease would be arbitrary and meaningless. There is little doubt that a dominant gene is involved in most family pedigrees with this disease, and a detailed discussion of the mode of inheritance of the disease will appear elsewhere.

Why some of the subjects will suffer severely while others will not is a question which cannot be answered with certainty. Possibly severe symptoms will develop only when access to water is insufficient. For example, increase in serum sodium concentration has been noted to occur when water intake is restricted. The degree of temporary derangement of renal function evidenced in Patient 1 of this series by reduction in urea clearance and renal base conservation has been observed in infants suffering from acute diarrheal disease of infancy. In three subjects studied prior to dehydration we have observed a profound reduction in urea clearance, base conservation, and even ability to produce a maximally concentrated urine.[25] What has been so impressive in Patient 1 is the remarkable recovery in renal function which occurred. The urea clearance and base-conserving powers which were so drastically reduced during his period of severe illness have returned completely to normal, as have also the concentrations in his serum of hydrogen ions, sodium, chloride, bicarbonate, and urea. Renal-function tests (aside from those measuring concentrating power) now fail to reveal any evidence of abnormality in this subject. This observation, coupled with those made on the other family members suffering from this disease, does not provide substantial evidence for the existence of renal disease as such. Furthermore, studies of kidneys of patients with this disease have failed to reveal any significant

structural abnormality.[4,26] The significance of the suggestion that there might be an abnormality in the convolutions of the tubules [10] is difficult to assess.

The term "nephrogenic" was coined after study of a patient, aged 34, in whom (aside from poor concentrating power) all studies revealed normal renal function, except for some diminution in renal plasma flow, urea clearance, and Tm iodopyracet. Since this patient also had essential hypertension and diabetes mellitus, the conclusion that these pieces of evidence for quantitative impairment of renal function tended to support the hypothesis that the diabetes insipidus was "nephrogenic" seems rather tenuous. On the contrary, it is most unusual to find corroborative anatomical or physiological evidence of renal disease in patients with vasopressin-resistant diabetes insipidus.

The increased amounts of total amino nitrogen excreted by these patients is remarkable. Patient 1 lost large amounts in his urine when he was severely ill, but subsequently, when he was in remission (except for polyuria and polydipsia), the amounts of amino nitrogen excreted were within normal limits. The increased amounts of amino nitrogen excreted by Patients 3 and 4 at times when they had no symptoms other than polyuria and polydipsia is noteworthy. One should not assume, however, that this finding supports the contention that there is a renal lesion since hyper-amino-aciduria occurs in rickets [22] and scurvy,[23] even in the absence of gross renal disease. In addition, hyper-amino-aciduria is not found in glomerulonephritis and pyelonephritis.[24]

The quantities of ammonia and titratrable acid excreted by these subjects after three days of ingestion of 6 gm. ammonium chloride per square meter per 24 hours fall within the normal range for children, according to studies which we have made in our own laboratory. In children over 18 months of age, after an adequate period of dehydration, the urine osmolarity should normally exceed 1,100 mOsm. per liter. All our patients therefore may be presumed to suffer from impairment of concentrating power. It may be pointed out that the urine osmolarity alone may serve to distinguish pseudodiabetes insipidus from true diabetes insipidus. In diabetes insipidus, in which vasopressin is absent, the distal nephron is presumably unable to elaborate hypertonic urine from the isosmotic fluid reaching it from the proximal nephron. As a result, the urine osmolarities in these patients are invariably less than 300 mOsm. per liter.[27] In pseudo diabetes insipidus, on the other hand, hypertonic urine can apparently be formed. We believe that Patient 7, who is upset about her disease and her needs for large amounts of water, gained access to water during the period when her physicians believed she was abstaining.

In regard to therapy, it is our belief that little will be gained by attempting to reduce the excreted osmolar load in these patients by dietary restrictions, since they may already have growth problems. It seems more reasonable simply to allow them free access to water. Certainly, Patient 1 in our series has grown well once he was able to shake off his infections and weather the period when his renal function was presumably immature. We have not advised any dietary restriction in his case.

Summary

A family is described in which there are eight patients who suffer from diabetes insipidus which is resistant to vasopressin.

Some of the patients suffer only from polyuria and polydipsia, but in one patient there occurred, temporarily, growth retardation, hypernatremia, reduced urea clearance, and severe impairment of renal powers of base conservation. In three of the patients there was marked hyper-amino-aciduria.

At the time of writing (aside from amino-aciduria and failure to concentrate maximally), renal function appears to be normal in all the subjects.

Evidence is presented to support the contention that, in many instances, at least, the disease is not transmitted by a sex-linked recessive gene but by an autosomal dominant.

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Hypnosis as an Adjunct to Anesthesia in Children

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Most children anticipate the prospect of submitting to surgery and anesthesia with fear and apprehension. Youngsters who have to be given an anesthetic are afraid that something disastrous may happen to them while they are asleep. The child fears not only what he can understand but also what is less understandable, such as will he ever wake up or is he going to be killed? There are many connections in the child's mind between anesthesia, sleep, and death.[1]

When a child is brought to the operating room heavily premedicated or asleep, without having his fears allayed or his anxieties resolved, he will usually experience a nightmare of restlessness on recovery. During such a confused and stressful state, a child is more subject to psychic trauma than one who will recover quickly and quietly to a state of full consciousness and awareness.[2] Sleep disturbances, temper tantrums, hallucinatory phenomena, and dependency reactions have been reported after surgical procedures where anesthesia was administered.[3] In order to cope with this situation, anesthesiologists in recent years have become more aware and better oriented to the psychological aspects of anesthesia for children.[4,5]

It is but a short step in inducing anesthesia in children with the use of stories, music, and flavoring agents to the superior effectiveness of an hypnotic induction. With children, relaxation by means of hypnosis is worth while. The comparative ease of inducing hypnosis in many children is a surprising finding. More than half of them can reach a sufficient hypnotic depth to permit minor surgical procedures, such as venipuncture, spinal tap, and reduction of simple fractures, to be performed.

Hypnosis is a valuable method of inducing anesthesia or achieving basal anesthesia in children.[6,7] Hypnotic inductions permit the use of posthypnotic suggestions that render recovery from anesthesia less distressing and more pleasant. Results in such cases are gratifying, because on recovery these children are quieter, more comfortable, and more cooperative than those who undergo anesthesia induced by chemical agents alone.

Although hypnosis may be performed successfully in younger children on occasion, youngsters between the ages of 7 and 14 years are the most susceptible. These children usually make good subjects because of their heightened powers of imagination and their ability to play a role or create a fantasy. I try to use the child's own experiences—things with which he is familiar, so that better imagery can be produced.

If children are admitted the day prior to surgery, a visit may be made in the afternoon or evening in order to take advantage of the opportunity of becoming acquainted with the child and to ascertain his area of interests. If it is possible to have a preliminary rehearsal of the hypnotic induction, it will serve the anesthesiologist well. However, no attempt should be made to force the situation. For children admitted in the early morning for surgery a few hours later or for emergency surgery, preliminary interview can usually be held in the operating room only. Light premedication is given so that consciousness may not be too clouded and response to suggestion may be possible.

A simple technique of indirect suggestion has been most successful. For example, it

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has proved to be invaluable in inducing anesthesia for tonsillectomy in over 50 cases. It makes induction of anesthesia smoother and helps to eliminate psychic trauma.

Prior to induction, the child's interests and likes and dislikes are ascertained. Most children enjoy television, although some like movies or sports better. I have found the "television game" most effective. Many youngsters like "Lassie." The child is asked to close his eyes and imagine he is viewing a television screen. When he can visualize the television set, he is asked to turn it on in his imagination and to raise his hand when the screen comes into view. When this is accomplished, verbalization of the hypnotic induction is begun with varying but repetitive suggestions of tiredness, deep breathing, and sleep. ("Lassie is breathing very deeply and feeling oh so tired, so very very tired, so tired that she is becoming drowsy, so very drowsy, that she is falling asleep. Now Lassie is going to take a long nap, and she is going to remain asleep, breathing deeply, until she is awakened.") This is what the child in his imagination sees Lassie do and what the child is also doing at the very same time, responding to the same indirect suggestion. Posthypnotic suggestions of well-being and comfort are now given. Also suggestions of being able to swallow readily and without discomfort are given. The induction of ether anesthesia is then begun with the following verbalization: "Lassie breathes deeply because there is a wind blowing across her face," and so does the child breathe deeply. Anesthesia is induced smoothly and rapidly, and surgery can then be performed without anesthesiological difficulty. At the termination of the surgical procedure the posthypnotic suggestions of well-being and postoperative comfort are again repeated. These posthypnotic suggestions are usually carried out with a great degree of accuracy. It should always be remembered that suggestions of sleep must be removed and the command to awaken directly or indirectly must be given.

Hypnosis also can be used in children as an adjunct to regional anesthesia. The following case report will demonstrate its successful use with caudal block.

Report of a Case

CASE 1.—A 13-year-old boy was brought to surgery for transurethral resection of the bladder neck because of a bladder neck contracture due to fibrosis, which resulted in repeated bladder infections. No premedication was given. The boy was hypnotized, and a caudal block was then performed using 15 cc. 0.5% lidocaine. Transurethral resection of the bladder neck was performed by using a No. 16 Resectoscope. No catheter was placed into the bladder postoperatively deliberately. The boy was then given the posthypnotic suggestions that he would have no pain and that he would be able to urinate freely and without discomfort. He was taken to the recovery room after surgery, where he was asked to awaken. He sat up smiling and cheerful, asked what time it was, and requested seeing another television program. On return to the pediatrics ward, he did watch actual television and had his full supper shortly thereafter. He voided spontaneously and without complaint. He slept soundly all night and ate a hearty breakfast the next morning. He was discharged from the hospital in good condition the following day.

Anesthesia for chest surgery in children requires the use of the least toxic medication and the lightest plane of anesthesia. This is especially true for those undergoing cardiac surgery. Whenever possible, hypnosis should be combined with chemical anesthetics in order to reduce toxicity and to minimize the dangers of cardiorespiratory depression as well as to obtund psychic trauma.[8] In open-heart operations for the surgical correction of congenital and acquired cardiac defects, the anesthesiological problems are manifold. Operative procedures involving the use of mechanical pump oxygenators are still hazardous and fraught with many complications. Yet this is the newest vista in cardiac surgery, and definite progress is being made.[9-11] I am reporting here the cases of two children on whom successful open cardiotomy was performed with the use of a mechanical pump oxygenator, employing hypnoanalgesia as the anesthetic technique. This is the first time in the annals of surgery and anesthesiology that such procedures were carried

out under hypnosis. The use of hypnosis was decided upon in order to avoid the depressant effects of anesthetic drugs and in an attempt to eliminate unconsciousness while on the pump oxygenator. It was also hoped that if consciousness could be demonstrated, it would help establish the pump oxygenator more firmly as a safe temporary prosthetic for the heart.

Report of Cases

CASE 2.—A 13-year-old boy weighing 88½ lb. was admitted to the hospital for the correction of a congenital infundibular stenosis of the heart. After serious consideration, it was decided to perform the surgery by means of open cardiotomy, with use of a mechanical pump oxygenator for the maintenance of an extracorporeal circulation. The boy was interviewed the day prior to surgery and agreed to play an "imagination game" with me. He reached a deep hypnotic level readily and was given the posthypnotic suggestion that the next morning he would go to sleep quickly and deeply at the signal command of "sleep now, sleep deeply." No medication was given for sleep that night, but the child slept soundly all night. At 6:30 a. m. the morning of surgery, he was given pentobarbital (Elixir Nembutal) 50 mg. and diphenhydramine (Elixir Benadryl) 10 mg. He was taken to the operating room at 8:30 in a calm relaxed state. Hypnosis was again induced by the signal command, "sleep now, sleep deeply," and after further suggestions a deep hypnotic level was obtained. An intravenous injection of 5% dextrose in water was begun in the right forearm. After five minutes of breathing 100% oxygen, 100 mg. thiamylal sodium was injected intravenously. To facilitate intubation, the larynx was sprayed with 5% hexylcaine topical solution and 10 mg. succinylcholine was given intravenously. A 32 F. oral endotracheal tube was inserted with ease and the inflatable cuff inflated. By means of vascular cutdown, cannulization with plastic catheter was performed in the left femoral and right radial arteries and the right saphenous and right brachial veins. Bilateral thoracotomy was performed, and total cardiac by-pass was carried out for 35 minutes with the child on the pump oxygenator, while a right ventriculotomy lasting 19 minutes was performed and the infundibular stenosis was removed. Total time on the operating table was six hours. The child was awakened when the operation was ended after posthypnotic suggestions regarding deep breathing, coughing on command, and absence of pain and discomfort postoperatively were given. He was talking when the endotracheal tube was removed shortly thereafter. The most remarkable postoperative aspect of this case was the absence of complaint of pain. The boy remained placid, calm, relaxed, and cooperative throughout the postoperative course and was discharged on the 30th day in good condition.

CASE 3.—A 14-year-old girl weighing 104 lb. was admitted to the hospital for surgical correction of a congenital subvalvular stenosis of the pulmonary valve. She was also afflicted with a congenital bilateral loss of hearing. This deafness handicap was partially overcome by the use of a hearing aid and by lip reading. Because of the successful experience in the previous case, it was decided to attempt an even greater depth of hypnosis on this patient and if possible to have her awaken at intervals while she was on the cardiac by-pass with the pump oxygenator, in order to evaluate possible brain damage while on the machine. This girl was interviewed three days prior to surgery and was found to be an excellent lip reader. This, of course, posed the question of communication when her eyes were closed. In order to solve this problem, a stethoscope was placed in the patient's ears and an image-projection technique involving a "radio broadcast" was used to induce hypnosis. She responded very well to suggestions "broadcast" through the chest piece of the stethoscope. Suggestions were given regarding sleep and ability to relax more quickly and more easily at subsequent sessions. During the next interview she complained of discomfort in the ears from the stethoscope and asked to wear her hearing aid. This was used for all subsequent sessions. On the morning of surgery, she received diphenhydramine 10 mg. by mouth and was hypnotized in her room. An 18 gauge needle was placed in her vein, and an infusion of 5% dextrose in water was begun. She was then taken to the operating room in a deep trance state. After she was placed on the operating table and after she had breathed 100% oxygen for 10 minutes, 100 mg. thiamylal sodium was injected intravenously. Endotracheal intubation was performed orally by direct laryngoscopy using a No. 9 Magill plastic endotracheal catheter. Succinylcholine, 10 mg., was given to facilitate intubation. Throughout the operation 100% oxygen was administered. Cutdown was performed in the right antecubital and right femoral veins and right radial and left femoral arteries. Bilateral thoracotomy was performed. Ventriculotomy was performed with use of the pump oxygenator to maintain circulation. At the time the heart was open, the hearing aid was placed in the child's ear and turned on. She was asked to open her eyes, which she did immediately. She was asked to move her head to indicate that she could hear me. This she also did. This response to command was elicited several times to satisfy the curiosity and amazement of the ob-

servers in the operating room. The subvalvular stenotic area was incised and partially excised, correcting the defect. When the heart was closed eight minutes later, she was given the command to go to sleep again. Total operating time was five hours. When extubation was performed, she was told to awaken, which she did immediately. She asked, "Is everything O. K.? May I have some water? I'm thirsty." She was given a few sips of water, which she retained. She was then taken to the recovery room in good condition. She was discharged from the hospital in good condition on the 20th postoperative day.

Both children have returned to school and are getting along well.

Summary

Although hypnosis has not had widespread employment in anesthesiology, there are increasing indications for its use.[12]

Hypnosis is a valuable adjunct to anesthesia in children. It may be employed successfully in the induction of anesthesia to make the course of anesthesia smoother and free from psychic trauma. It can also be used with benefit in conjunction with regional anesthesia. Children usually make ideal subjects for hypnosis. Those from ages 7 to 14 years are the most susceptible, although hypnosis may be effective in the younger age group.

The advantages of hypnosis are found in the use of reduced doses of chemical agents and in the effective use of posthypnotic suggestion which makes for a more pleasant reaction from anesthesia and a smoother postoperative course. The primary disadvantage of hypnosis is the additional time required, which may not always be available in a busy practice.

The first two cases using hypnoanalgesia as the anesthetic technique for cardiac ventriculotomy are described. These cases reveal that hypnosis raises pain thresholds and that consciousness is not lost under hypnosis.

Conclusion

Successful hypnosis as a means of or adjunct to anesthesia helps eliminate the depressing effects of prolonged chemical anesthesia. Hypnosis is a useful method, which should be added to the armamentarium of pediatric anesthesia.

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Bacterial Flora of the Nasopharynx in Children

Influence of Respiratory Infections and Previous Antimicrobial Therapy

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The high incidence of respiratory tract disease in children, coupled with the rather indiscriminate use of antimicrobial agents, stands in marked contrast to the paucity of exact data concerning the bacterial flora of the nasopharynx. The frequency of the development of resistant organisms which may lead to serious complications indicates that such data should be obtained. The purpose of the present communication is to report the results of the nasopharyngeal cultures from 1,116 infants and children.

Material and Methods

Thirteen hundred eighty-five cultures on 1,116 children between the ages of 3 weeks and 14 years were obtained in the outpatient clinic of the Children's Hospital of the University of Texas Medical Branch during the period from October, 1952, through July, 1954. The vast majority of cultures were from subjects having mild or severe respiratory tract symptoms, while 104 were from infants and children having no evidence of respiratory disease.

Cultures were taken from the nasopharynx by the use of very small cotton swabs prepared on flexible stainless steel wire. By insertion of the wire through the nares, the cotton was allowed to rest against the posterior pharyngeal wall for about 30 seconds. Specimens were sent to the pediatric bacteriology laboratory, where they were planted on appropriate media.

The base used for all agar plates was Casman Medium Base (Difco) containing 7% human blood. Each swab was planted on a blood-agar plate and a chocolate-agar plate, which were incubated in sealed candle jars at 37.5 C for 24 hours. After readings were made, the plates were reincubated under normal atmospheric conditions for an additional 24 hours. The amount of growth of the organisms was recorded as heavy, moderate, light, or none. Identification was made by growth requirements, colony morphology, Gram stain, hemolysis, type of growth in heart-infusion broth, bile solubility, and pigment production. Plate-sensitivity tests with use of disks and tablets were performed for the predominating pathogens, with Trypticase Soy Agar (BBL) as the base for the blood and chocolate plates. Although a great variety and multiplicity of organisms were isolated, including Neisseria, corynebacteria, nonhemolytic streptococci, and Gram-negative bacilli, special attention was given to the following five organisms: Diplococcus pneumoniae (pneumococcus), α- and β-hemolytic Streptococcus, Staphylococcus pyogenes var. aureus, and Hemophilus species. Data concerning these pathogenic organisms were tabulated for statistical examination with the assistance of Dr. J. Allen Scott, of the Department of Preventive Medicine and Public Health.

The information on the laboratory report cards and the patients' charts was transferred to marginal punch cards on which were recorded the patient's name, unit history number, age, date of visit, diagnosis, medication within the 24 hours prior to culturing, treatment used, and organisms present, as well as the amount of growth. A new card was prepared for each attack of respiratory disease. After all the cards had been completed and the various factors had been sorted against each other, charts and tables were prepared.

The patients were grouped into three categories based on the diagnosis entered on the chart by a member of the pediatric staff. These were as follows:

GROUP I.—Mild upper respiratory infection: tonsillitis, pharyngitis, laryngitis, bronchitis, sinusitis, and otitis media.

GROUP II.—Severe respiratory infection: laryngotracheobronchitis, bronchiolitis, bronchopneumonia, and lobar pneumonia.

GROUP III.—No respiratory infection: control subjects with such diagnoses as gastroenteritis, chronic nephritis, inactive rheumatic fever, feeding problem, and behavioral disturbance.

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Results

Relative Incidence of Organisms.—The incidence of the five organisms in Groups I, II, and III, regardless of whether the patients had received any antibiotic or chemotherapeutic medication prior to culture, is shown in Figure 1. Similar data concerning the same organisms in the children who had had no previous medication are presented in Figure 2. The pattern of distribution of the organisms is strikingly similar. In all groups D. pneumoniae were isolated in a higher percentage of cultures than were any other bacteria, the highest incidence being in the group suffering from severe respiratory infection. There was little or no difference among the groups with respect to the isolation of α- or β-hemolytic streptococci. In both figures it is observed that S. pyogenes var. aureus was present in a much higher percentage of cultures from children with no respiratory tract infection (Group III) than from those with respiratory tract symptoms (Groups I and II). Hemophilus species were isolated more frequently in Groups I and II than in the control subjects (Group III).

Tests for significance of the findings were performed with use of χ^2 and taking a value of P at 0.05 or less as statistically significant. The results were as shown in Table 1. It is obvious that previous antimicrobial therapy decreased the incidence of pneumococci. In reference to S. pyogenes var. aureus, there is likewise a statistical difference; however, this is due to a greater number of isolations from the children who had been given antimicrobial therapy. The isolations of Hemophilus species in the ill children (Groups I and II) are not significantly greater than in the control children (Group III). There is no difference among the groups with regard to the isolations of α-hemolytic streptococci.

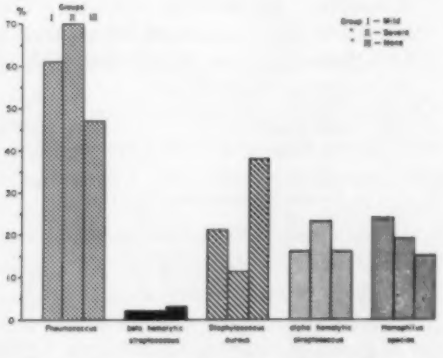

Fig. 1.—Incidence of organisms in 1,385 nasopharyngeal cultures from patients with mild, severe, and no respiratory tract symptoms.

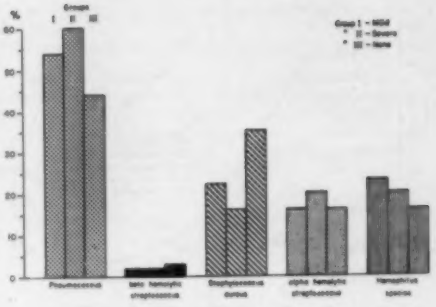

Fig. 2.—Incidence of organisms in 1,099 cultures from patients with mild, severe, and no respiratory tract symptoms who had had no previous medication.

TABLE 1.—*Results of Significance Tests in Relative Incidence of Organisms*

	Pneumococci		S. Pyogenes Var. Aureus	
Group	All Cultures	No Previous Medication	All Cultures	No Previous Medication
I & II	------	------	------	<0.01
I & III	>0.05	<0.01	>0.75	<0.001
II & III	<0.01	<0.001	<0.001	<0.001

Seasonal Variations.—The incidence of organisms isolated (October-December, January-March, April-June, July-September) in the subjects in Group I who had had no medication within 24 hours prior to culturing is shown in Figure 3. The highest percentage of isolations of D. pneumoniae occurred during the October-December quarter in both years (73% and 69%, respectively). The number of isolations in

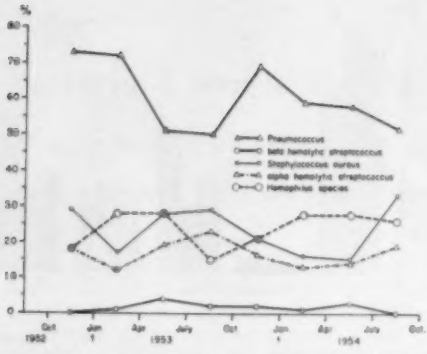

Fig. 3.—Seasonal incidence of organisms in 882 cultures from patients with mild respiratory tract symptoms; no previous medication.

the January-March quarter was somewhat less, especially during 1954. The lowest incidence in both years occurred in July-September (50% and 52%). Rather remarkable was the low incidence of β-hemolytic streptococci for the entire period. This ranged from 0% to 4%; however, a seasonal trend may be observed in that the highest percentage in both years occurred in the spring (April-June) quarter. The seasonal variations in S. pyogenes var. aureus and α-hemolytic streptococci closely paralleled each other. Both occurred least frequently during January-March in 1953 and January through June in 1954. A tendency to a reverse pattern is shown for Hemophilus species, which were isolated most frequently from January through June. The lowest incidence of occurrence during 1953 was from July through September.

Age in Relation to Isolation of Organisms.—The distribution of organisms according to age for the children in Group I who had had no previous medication is shown in Table 2. Pneumococci were isolated with similar frequency (62% to 65%) in children between the ages of 3 weeks and 2 years and with somewhat lower frequency in the older children (2-6 years, 59%; 6-14 years, 50%). The incidence in children over 6 years of age is significantly lower than in the younger children ($P = <0.01$). The number of instances wherein β-hemolytic streptococci were isolated was so small as to make evaluation impossible. S. pyogenes var. aureus occurred in 38% of the youngest infants; it decreased remarkably after 3 months but tended to rise after the age of 2 years. The incidence in the youngest and in the oldest subjects is significantly different from that in the other children, respectively, $P = <0.001$ and $P = <0.005$. The highest incidence (21%) of α-hemolytic streptococci occurred in those between 6 months and 1 year and the lowest (12%), between 3 and 6 months. Hemophilus species occurred in an average of 24% of the cultures from all children under 6 years of age. The highest incidence was from 6 months to 1 year (28%) and the lowest from 3 to 6 months (18%). After the age of 6 years the incidence was lower than in all the other children ($P = <0.05$).

Magnitude of Bacterial Growth.—The incidence of heavy growth of the organisms where there had been no previous medica-

TABLE 2.—*Relation of Age to Incidence of Organisms in 882 Cultures from Patients with Mild Untreated Respiratory Tract Symptoms*

Age Group	Cultures, No.	Pneumococcus		β-Hemolytic Streptococcus		S. Pyogenes Var. Aureus		α-Hemolytic Streptococcus		Hemophilus Species	
		No.	%*	No.	%	No.	%	No.	%	No.	%
3 wk. - 3 mo.	53	33	62	2	4	20	38	10	19	11	21
3-6 mo.	75	47	63	0	0	13	17	9	12	14	18
6 mo. - 1 yr.	157	102	65	4	3	22	14	33	21	44	28
1-2 yr.	213	138	65	3	1	37	17	28	13	32	25
2-6 yr.	273	161	59	4	1	61	22	45	17	71	26
6-14 yr.	111	55	50	3	3	35	32	16	14	18	16

* % of cultures positive, e. g., 33=62% of 53.

TABLE 3.—*Magnitude of Growth in Relation to Severity of Disease—No Previous Antimicrobial Therapy*

	Group I—Mild Upper Respiratory Tract Symptoms			Group II—Severe Respiratory Tract Symptoms			Group III—No Respiratory Tract Symptoms		
		Heavy Growth			Heavy Growth			Heavy Growth	
	No. Isolated	No.	% *	No. Isolated	No.	%	No. Isolated	No.	%
Pneumococcus	539	303	56	80	50	62	45	13	29
β-Hemolytic streptococcus	16	5	31	1	1	100	3	1	33
S. pyogenes var. aureus	189	43	23	14	1	7	36	16	44
α-Hemolytic streptococcus	143	54	38	28	7	25	15	2	13
Hemophilus species	210	73	35	23	11	48	14	0	0

* % of isolations occurring in heavy growth, e. g., 303=56% of 539.

tion is shown in Table 3. Pneumococci were isolated in heavy growth twice as frequently when disease was present as when no respiratory tract infection existed. This difference is highly significant ($P=<0.001$). The difference for those moderately ill and those severely ill is not significant ($P=>0.30$). The number of isolations (44%) of a heavy growth of S. pyogenes var. aureus was higher in the control subjects (Group III) than in the sick children (Groups I and II) ($P=<0.005$). The difference between Group I and Group II is not significant ($P=>0.10$). The abundant growth of α-hemolytic streptococci was two to three times more frequent when respiratory tract infection was present. Remarkable, indeed, was the failure to isolate Hemophilus species in heavy growth in Group III, whereas the incidence was 35% in Group I and 48% in Group II.

Influence of Previous Antimicrobial Medication.—The relative incidence of organisms in the 1,281 cultures from children having respiratory tract disease, with and without antimicrobial medication during the 24 hours previous to culture, is depicted in Figure 4. The incidence of D. pneumoniae was less than half in the treated subjects ($P=<0.001$). The total incidence of isolations of β-hemolytic streptococci was very low; however, it is rather remarkable that 19 of the 21 positive cultures occurred in subjects who had had no previous medication. S. pyogenes var. aureus isolations

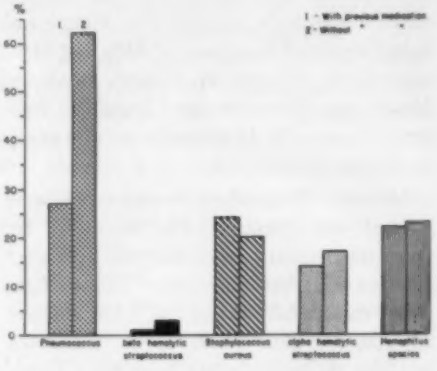

Fig. 4.—Incidence of organisms in 1,281 cultures from patients with respiratory tract symptoms, with and without previous medication.

were somewhat greater if antimicrobial therapy had been given. The occurrence of positive cultures of Hemophilus species was unchanged.

Comment

Influence of Bacteriologic Techniques.—Differences in techniques employed make it difficult to compare findings among various authors [1-5] regarding the bacterial flora of the nasopharynx. One factor which may affect the results is the care with which the specimen is obtained. Another is the type of procedure followed, for example, the use of swabs from wire or wooden applicators or a platinum loop may give different results. Also, the route of introduction of the swab, whether peroral or pernasal, and the distance it is inserted into the nasopharynx, as

well as the length of time it is allowed to remain in place, may be important in the isolation of pathogenic bacteria. If the swabs are planted on blood-agar plates only, it is very possible for minute colony growth of Hemophilus influenzae to be overlooked. Chocolate-blood-agar supplies sufficient of the heat-stable "X" factor to yield a good growth of this organism.

Comparative data are few concerning the bacterial flora of the nasopharynx in children. Although it seemed that the 47% incidence of pneumococci in our control children was high, we find that Yang [1] and Rabe [2] reported incidences of 44% and 34%, respectively. On the other hand, Goldman, Bloom, and Herschberger [3] found an incidence of only 2% D. pneumoniae in a group of 50 normal children.

Although β-hemolytic streptoccoci were isolated infrequently (2% to 3%), the range was similar to that observed by Yang [1] (2% to 5%) and Goldman [3] (2%). Rabe found this to be 10% in the 1,356 children reported by him. There is the possibility that our incidence might have been higher had simultaneous throat cultures and enrichment broth been used.[6] However, by employing throat cultures Teberg and Adams [7] reported a 2% to 6% incidence and Breese and Disney [8] reported 2%. On the other hand, Pike and Fashena [4] in a study of 900 throat cultures found an incidence of 42% β-hemolytic streptoccoci when enrichment broth was used but only 14% by direct plating. Rantz, Maroney, and DiCaprio [5] reported that throat-swab cultures in children, with use of a wooden applicator, yielded a much higher percentage of this organism than nasopharyngeal cultures obtained with a wire swab.

At first thought it seems unusual that there was a higher incidence of S. pyogenes var. aureus in the control subjects than in those severely ill with respiratory tract disease. However, when one considers the reputation which this organism has gained as a saprophyte, perhaps this finding is not so surprising. Nevertheless, whether the same difference would be evident were one to carry out bacteriophage typing for S. pyogenes var. aureus it is impossible to say. In view of the increasing recognition of certain types of S. pyogenes var. aureus as being highly pathogenic and responsible for a number of serious epidemics, greater consideration must be given to typing of this organism. It has been shown by a number of investigators [9-11] that S. pyogenes var. aureus is isolated much more frequently from the anterior nares than from the nasopharynx.

All Hemophilus species isolated in this study were probably either H. influenzae or H. parainfluenzae, as they grew much more luxuriantly on chocolate agar than on blood agar, and in most instances growth on blood agar was dependent upon the presence of other organisms, including staphylococci, D. pneumoniae, Neisseria, or diphtheroids. H. hemolyticus, which is recognizable by β-hemolysis on blood agar and by Gram stain, was not isolated from any of the cultures. The fact that Hemophilus species were isolated in 20% to 30% more cultures from ill children than from well children would seem to imply a relationship between these organisms and disease. However, strains were not subjected to specific typing with Type a and Type b antisera. The prevalence of H. influenzae meningitis indicates that it would be desirable to ascertain the incidence of the various types of this organism in a childhood population. In 1943, Alexander [12] reported that about 25% of nasopharyngeal cultures from 847 unselected patients were positive for H. influenzae. Of the strains isolated, 19% were typable and 81% were nontypable. Of the typable strains isolated from children with no respiratory tract infection, 16% were of Type b and of those isolated from children with infection, 29%.

Season and Age.—The prevalence of D. pneumoniae during October through March coincides with the period in which most of

the respiratory tract infections usually occur in this area. The appearance of β-hemolytic streptococci was most frequent in the spring. A similar trend has been found to occur on the eastern seaboard, which incidence coincides with the period of greatest frequency of scarlet fever and streptococcal sore throat.[13] Wilson [14] stated that the seasonal pattern for the appearance of β-hemolytic streptococci was the same for normal carriers as for infected persons. In New York City the incidence was found to be higest from February through June and lowest from September through January, while Pike and Fashena,[4] in Dallas, found no particular seasonal variation. The reason that the incidence of S. pyogenes var. aureus and α-hemolytic streptococci should parallel each other so closely is not understood. When these two organisms were most prevalent, Hemophilus was found less often. One naturally wonders about bacterial as well as viral synergism and antagonism. As is well known, the synergism of S. pyogenes var. aureus and Hemophilus is often helpful or essential in vitro in the recovery of Hemophilus from certain specimens. The level for isolations of Hemophilus species remained high constantly for the first six months of the year. This high level was attained three months later than the high level for pneumococci and persisted three months longer (Fig. 3). Alexander [12] observed no seasonal variation in the prevalence of H. influenzae.

Data from the control subjects were too few to evaluate isolation of any of the organisms in relation to age. Children between 6 and 14 years of age with respiratory tract infection were found to have fewer D. pneumoniae than those in the younger age groups. Hemophilus species were isolated most frequently from children between the ages of 6 months and 1 year, which age group has the highest incidence of H. influenzae meningitis.[15] The much higher incidence of S. pyogenes var. aureus in the youngest age group than in the other groups, up to the age of 6 years, is not surprising in view of the fact that this organism is found to be present in the anterior nares and nasopharynx in a high percentage of newborn infants within the first few days of life.[16-18] A similarly high incidence has been found in hospital personnel.[19-21] At the time this study was undertaken the seriousness of staphylococcal infection was scarcely appreciated. It was not until two years after this study was completed that the magnitude of this problem was forcibly brought to our attention.[22] It is axiomatic that henceforth phage typing of the S. pyogenes var. aureus be carried out in the attempt to control these epidemics.

Magnitude of Growth and Effect of Previous Medication.—D. pneumoniae and Hemophilus species in heavy growth were associated frequently with respiratory disease states. On the other hand, S. pyogenes var. aureus was more frequently found in abundant growth in the absence of infection of the respiratory tract.

The relative sensitivity of the various organisms is indicated in the comparison of isolations from patients with and without previous antimicrobial medication. As is shown in Figure 4, the greatest effect of previous medication is found in relation to the frequency of isolation of pneumococci. By in vitro tests the great majority of strains of this organism were found to be sensitive to most of the current therapeutic agents. In a previous report [23] concerning children with respiratory tract disease who had received antimicrobial therapy, the incidence of isolations was as shown in Table 4. It is apparent that the combination of penicillin

TABLE 4.—*Incidence of Isolations*

	Cultures, No.	Isolations, %		
		Pneumococcus	S. Pyogenes Var. Aureus	Hemophilus
No medication	882	61	21	24
Sulfisoxazole	76	32	28	14
Penicillin	61	26	28	23
Sulfisoxazole + penicillin	70	16	15	29

and sulfisoxazole was more effective in reducing the isolations of pneumococci than was either drug alone. These drugs in combination also decreased the percentage of cultures positive for S. pyogenes var. aureus, whereas the use of either drug alone seemed to cause an increase. Stoppelman [18] found in premature infants more pure cultures of S. pyogenes var. aureus among those receiving antimicrobial therapy than in those without treatment, although the incidence of positive cultures was increasingly high regardless of medication. The widespread incidence of S. pyogenes var. aureus remains a challenge to bacteriology as well as to modern hospitalization of newly born infants. Sulfisoxazole alone was most effective against Hemophilus species.

Summary

The incidence, over a two-year period, of five potentially pathogenic bacteria has been determined from a study of 1,385 nasopharyngeal cultures from 1,116 children who were seen in the outpatient clinic in Galveston, Texas. There were 1,110 cultures from children with mild respiratory tract infection, 171 from those with severe infection, and 104 from those with no disease of the respiratory tract. Where no previous antimicrobial medication had been received, the following incidence of the organisms was found in children having, respectively, mild, severe, or no respiratory tract symptoms: Pneumococci, 61%, 70%, 47%; β-hemolytic streptococci, 2%, 2%, 3%; Staphylococcus pyogenes var. aureus, 21%, 11%, 38%; α-hemolytic streptococci, 16%, 23%, 16%; Hemophilus species, 24%, 19%, 15%.

In children with mild respiratory tract infection pneumococci were isolated most frequently from October through March; β-hemolytic streptococci, from April through June, and Hemophilus species, from January through June. α-Hemolytic streptococci and S. pyogenes var. aureus had identical patterns of seasonal variation which, in general, were the reverse of the pattern for Hemophilus.

Pneumococci occurred more frequently in children under the age of 6 years, while Hemophilus species were most frequently recovered from infants between 6 months and 1 year of age. S. pyogenes var. aureus was isolated most frequently from infants between the ages of 3 weeks and 3 months.

A heavy growth of pneumococcus and of Hemophilus species seemed to be indicative of severe infection. S. pyogenes var. aureus, on the other hand, was found in heavy growth in infants without infection.

Previous antimicrobial medication greatly decreased the incidence of pneumococci in children with respiratory tract disease. Of the isolations of β-hemolytic streptococci, 19 of the 21 cultures occurred where there had been no previous medication. There was a slight increase in the incidence of S. pyogenes var. aureus in those who had had previous medication.

The results of this study seem to demonstrate the need for exact knowledge of the nasopharyngeal flora in children with respiratory tract symptoms, especially before antimicrobial therapy is instituted.

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The Nonspecific Inflammatory Cycle in the Neonatal Infant

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The failure of the newborn animal and human infant to exhibit the usual signs of inflammation in response to foreign substances is documented in the experimental and clinical literature. For example, Adelsberger,[1] Rachmilewitsch,[2] and Friedberger and Heim [3] showed that such exotic irritants as croton oil, mustard oil, or eel serum failed to elicit the usual violent inflammatory reaction when applied to the skin of the neonatal infant. Von Groër and Kassowitz [4] and Cooke [5] found that the Schick and Dick toxin reactions are suppressed in the young infant independent of the presence or absence of specific antitoxin in the serum. Similar observations in newborn animals have indicated not only that there is a failure of response to nonspecific irritants, but that bacterial or viral skin infections often fail to elicit the usual mature local response [6] leading to systemic dissemination of the agent. Evidence is also available that during the newborn period the stereotyped physiologic responses to infection by microorganisms or intoxication by their products,[7] is absent or minimal when compared to mature members of the species. It would appear that the mature pattern of local response to inflammation and of systemic physiologic response is gradually acquired during the early period of growth with temporal variations among different species.

It seemed an appropriate point of departure in investigating the mechanism of apparently impaired local and systemic reactive capacity, to examine in detail the nonspecific inflammatory cycle in the neonatal infant and in newborn animals, searching for any qualitative or quantitative defects which might characterize the immature inflammatory response. The studies to be reported in this paper are concerned with the nonspecific inflammatory cycle in the normal newborn human infant. Similar studies in experimental animals will be reported elsewhere.

Materials and Methods

The technique employed, a modification of that described by Rebuck and Crowley,[8] offered an effective and safe means of studying the various stages of inflammation at frequent intervals without serial biopsy of the skin. It is based upon the principle that leukocytes in an inflammatory exudate adhere to glass surfaces, and it involves the assumption that each morphologic category of leukocyte is equally available to and adhesive upon the glass.

Normal full-term infants 10 hours to 21 days of age who had no evidence of a skin rash or infection were studied. The older infants, children, and adults who were examined were either patients hospitalized for minor surgical procedures or members of the medical, nursing, or laboratory staff.

For the procedure, the skin was first cleaned thoroughly but gently with hexachlorophene (pHisoHex) followed by 70% alcohol and it was then gently dried with cotton pledgets. The superficial epidermis was scraped with a sterile No. 10 Bard Parker scalpel blade until a slightly abraded surface had been produced with minute areas which oozed serum. If scraping was too energetic, or the skin was inadvertently cut, resulting in frank

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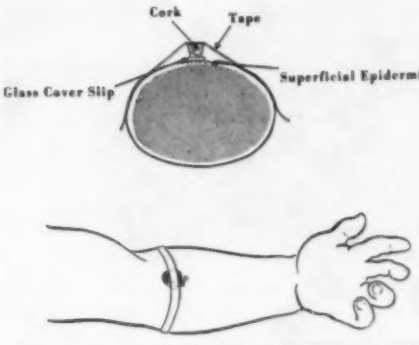


Fig. 1.—Schematic illustration of the orientation of the cover-slip and cork over the abrasion in a typical preparation.

bleeding, the area was unsatisfactory for study. A sterile thin cover glass (Arthur H. Thomas No. 7022) was placed over the abraded surface, which usually measured 1×1 cm. A cork which had been cut to fit the cover slip was then positioned directly over it to provide even pressure by the glass over the area of the abrasion. A piece of adhesive tape was then applied to hold the preparation in place and under slight pressure. The appearance of the completed preparation is schematically illustrated in Figure 1. Either the volar surface of the forearm or the back proved to be satisfactory and accessible. When serial studies were performed a new site was used on each occasion.

After the initial application, the cover slips were changed at one- or two-hour intervals using sterile technique. The cover glass, removed from the abraded area, had upon its surface a sampling of the leukocytes accumulated during the time interval it had remained in place. After rapid air drying the glass was mounted, exudate side up, upon a regular microscopic slide with Permount to facilitate identification and staining. The cellular exudate on the surface of the cover slip was stained with Wright and Giemsa stains, and differential cell counts were made of at least 500 cells. For purposes of this report the cells were classified only as granulocytes, eosinophils, or mononuclear cells, no attempt being made to differentiate between morphology of various categories of mononuclear cells. The typical appearance of each of these three categories of cells is illustrated in Figure 2 in order to clarify the remainder of the illustrations. Simultaneous replicate samplings at different sites on the same infant had a high order of agreement.

Results

Examination of the preparations made from older children and adults revealed a sequence of stereotyped changes in the cell population of inflammatory exudates similar in every way to those previously reported by Rebuck and Crowley.[5] The earliest cells to appear in the inflammatory cycle in those more mature subjects were polymorphonuclear neutrophils with rare mononuclear cells in evidence (Fig. 3*a-e*). In four-hour exudates, the cells still consisted of 85% to 95% polymorphonuclear neutrophils, the remainder being mononuclear cells. The proportion of mononuclear cells in the exudates then increased steadily during the next four hours; at eight hours these cells comprised 50% to 70% of the inflammatory exudate, and this distribution persisted at 10 and 12

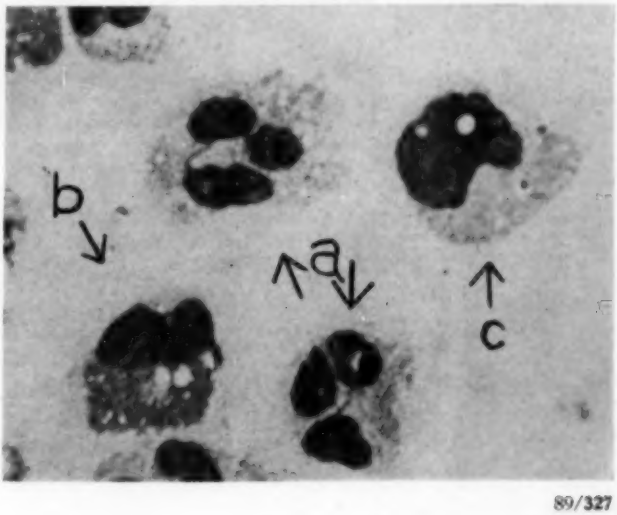


Fig. 2.—The morphological characteristics of the three general cell types encountered are shown in an early exudate in a 19-day-old infant. (*a*) Polymorphonuclear granulocyte; (*b*) eosinophil, the coarse granularity of the cytoplasm identifies this cell in the black and white reproduction; (*c*) an early mononuclear component of the exudate. Mononuclear cells in later exudates may appear vacuolated or contain granules.

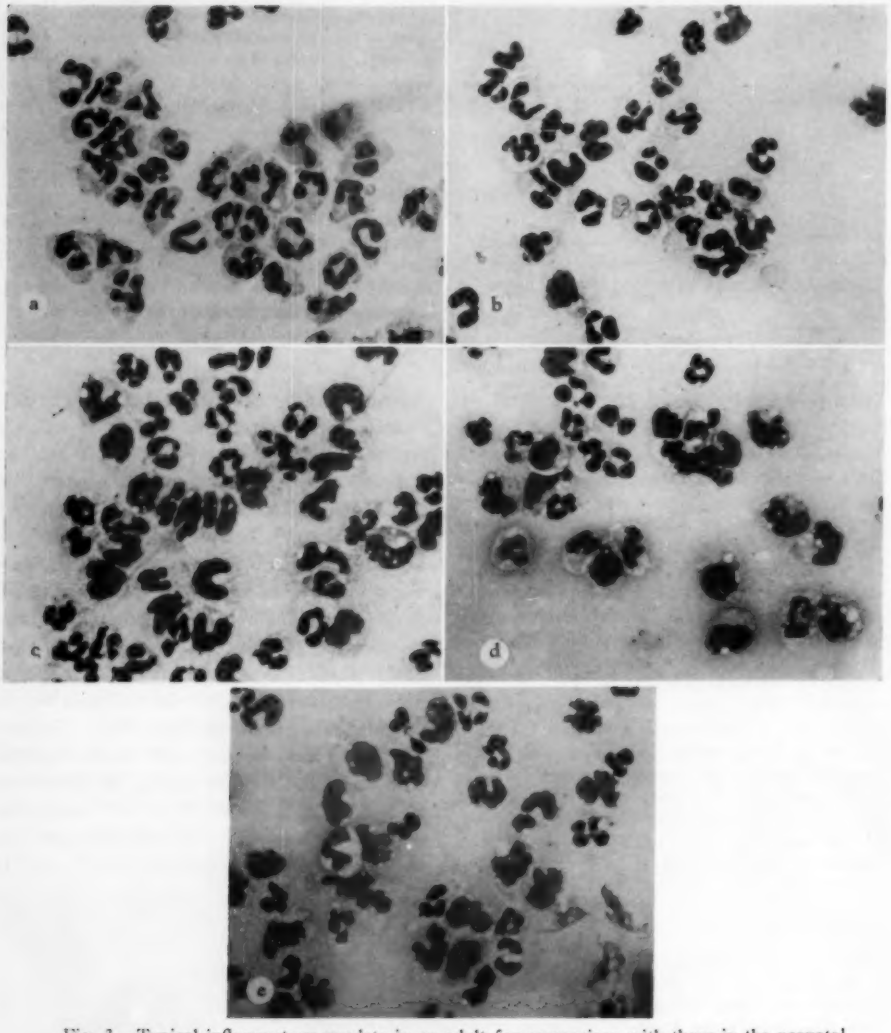


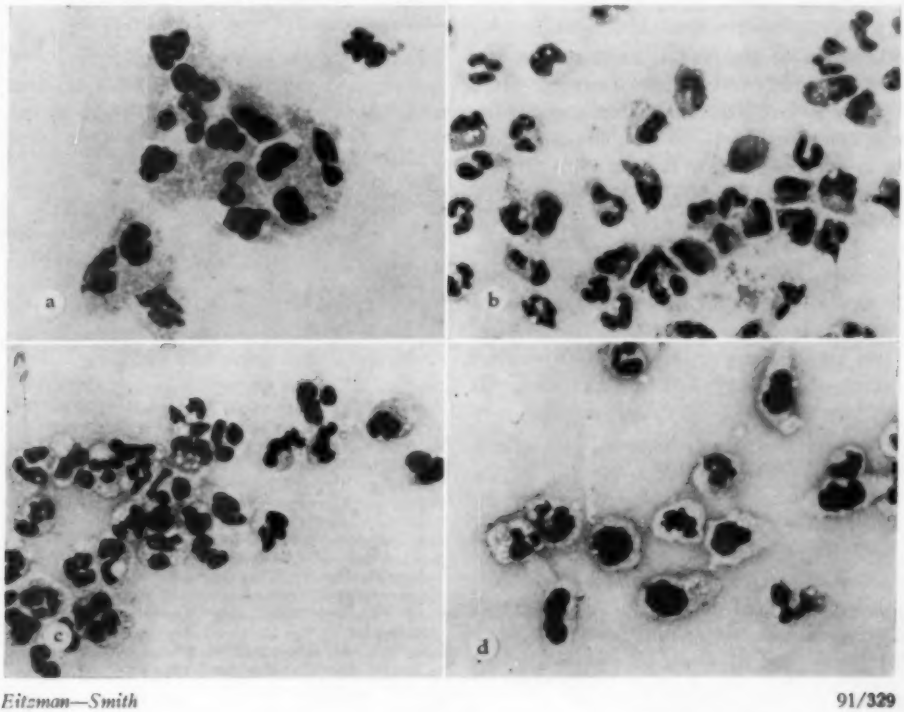
Fig. 3.—Typical inflammatory exudate in an adult for comparison with those in the neonatal infants. (*a*) Exudate at two hours with a cell distribution of 98% polymorphonuclear granulocytes and 0.8% eosinophils; (*b*) at four hours with 95% polymorphonuclear granulocytes and 0.5% eosinophils; (*c*) at six hours with 82% polymorphonuclear granulocytes and 0% eosinophils; (*d*) at eight hours with 67.5% polymorphonuclear granulocytes and 0.4% eosinophils; (*e*) at 10 hours with 55% polymorphonuclear granulocytes and 0% eosinophils. The remainder of the cell population not specifically listed was composed of lymphocytes and macrophages.

hours. From 6 to 12 hours (Fig. 3*d*, *e*) the mononuclear cells were often observed to become larger, the cytoplasm appeared more pale, and many cells developed multiple vacuoles and inclusions in the cytoplasm. Variations in this mature sequence from subject to subject were infrequently observed, although the time at which maximal shift to mononuclear cells occurred varied between 8 and 12 hours. Significant numbers of eosinophils were not found in any of the mature inflammatory exudates examined during the 12-hour period under study.

In the young infants two principal variations from the mature pattern were encountered. Most striking and most consistent was the finding of early inflammatory eosinophilia in the infants examined between 24 hours and 21 days of age. All but one examined in this age category were found to have accumulated varying proportions of eosinophilic granulocytes in their 2-, 4-, and 6-hour exudate samplings. This observation is illustrated in Figure 4(*a-d*) by photomicrographs of representative areas on the cover slips taken at two, four, six, and eight hours in an infant 3 days old.

The presence or absence of early inflammatory eosinophilia provided the basis for dividing the data into three age groups, i. e., those infants 0 to 24 hours of age who exhibited no inflammatory eosinophilia, those 2 to 21 days old who regularly showed early eosinophilia in excess of 2% of all cells, and those 21 days and older, all of whom exhibited the mature pattern. In the scatter diagrams presented in Figure 5(*a-d*), data on all persons examined were sorted into these three age groups, each point representing the proportion of eosinophils or granulocytes found in a sample of the inflammatory exudate at that particular time for one subject. At two hours (Fig. 5*a*) the exudate in all three groups consisted almost entirely of granulocytes. In the 2- to 21-day-old group, however, a variable proportion of the granulocytes, but exceeding 2% in all but one infant, were eosinophils. The greatest degree of inflammatory eosinophilia was found in each subject in this group at two hours, and the average

Fig. 4.—Example of samplings of exudates at two, four, six, and eight hours after the abrasion in a 3-day-old infant. Note the intense inflammatory eosinophilia, most marked in this infant at two hours but still evident at four hours. By eight hours the granulocytes present were chiefly neutrophilic. (*a*) Sample two hours after initiation of the inflammatory cycle with 6% polymorphonuclear granulocytes and 93% eosinophils; (*b*) at four hours there were 61.5% polymorphonuclear granulocytes and 31.5% eosinophils; (*c*) at six hours there were 78% polymorphonuclear granulocytes and 8% eosinophils; (*d*) at eight hours there were 65% polymorphonuclear granulocytes and 2% eosinophils.



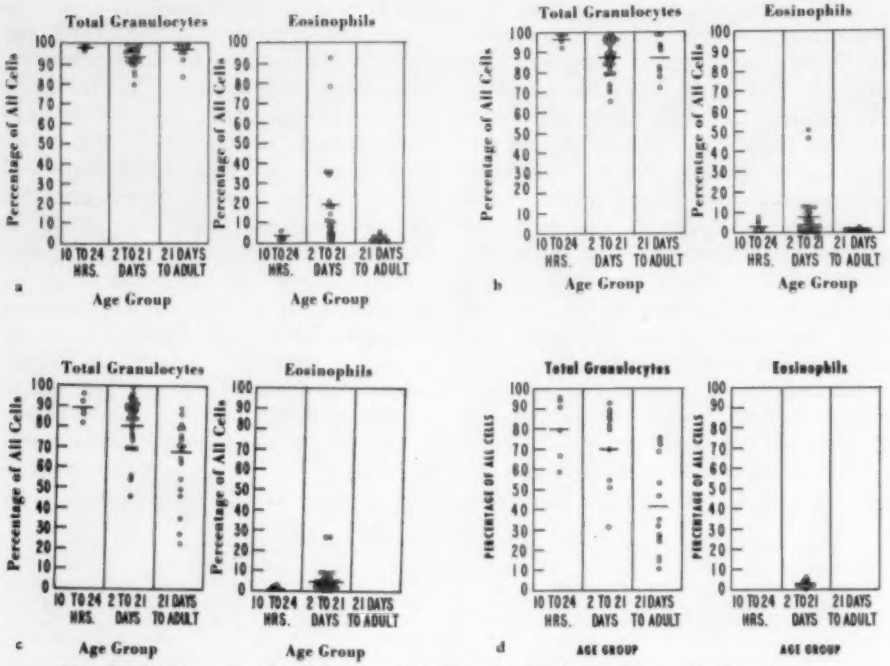


Fig. 5.—Scattergrams of individual exudate samples showing total granulocytes as the sum of the polymorphonuclear granulocytes and the eosinophils. (*a*) Cellular composition of inflammatory exudate at two hours; (*b*) at four hours; (*c*) at six hours; (*d*) at eight hours.

proportion of eosinophils at this time was 19% of the exudate cells, ranging from 1% to 93%. No comparable eosinophilia was found in the 0- to 24-hours-old group or in those older than 21 days (Fig. 6). At four hours (Fig. 5*b*) the percentage of eosinophils was lower; the mean value in the 2- to 21-day age group was 7%, compared to 0% and 2% in the other two groups. At six hours the proportion of eosinophilic granulocytes, even in those infants having intense early eosinophilia, had decreased markedly, and by eight hours this cell type was rarely observed.

To confirm the observation that inflammatory eosinophilia did not occur in the youngest infants, serial observations of the inflammatory cycle at different ages were made on several infants. In one of these studies, illustrated by the series of photomicrographs of four-hour exudates in Figure 7, observations were initiated at 15 hours, 31 hours, and 61 hours of age in a single infant. At 15 hours of age, very few eosinophils were found in the early exudate and the four-hour sample consisted almost

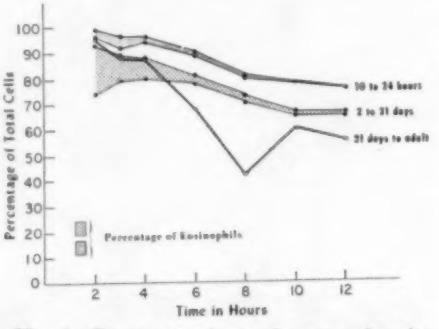


Fig. 6.—Percentage of granulocytes and eosinophils in the inflammatory exudate. Graph shows average value at each time of sampling, showing the difference between the three age groups. The top line for each group represents the total granulocytes and the cross hatched area between the two lines represents the percentage of eosinophils in the total exudate. Note the greater percentage of eosinophils in the 2- to 21-day age group. Also note that the percentage of granulocytes decreases after six hours as the age increases.

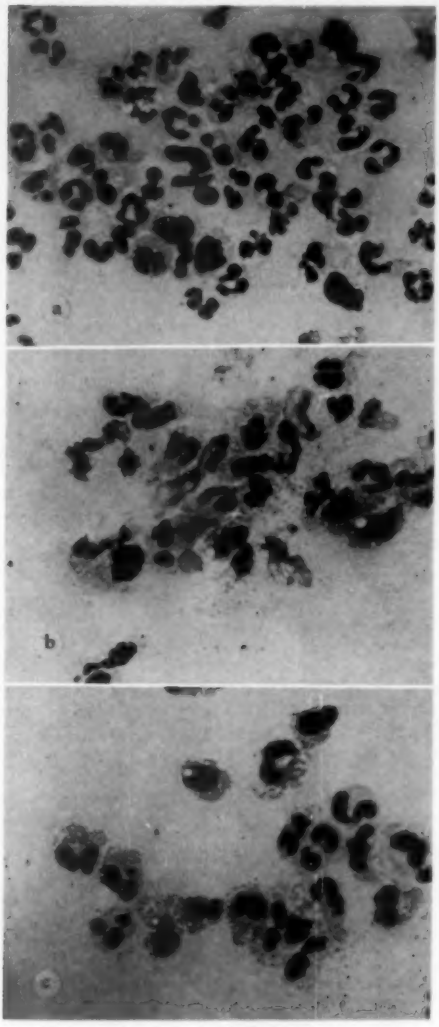


Fig. 7.—Inflammatory exudate from a newborn infant when the inflammatory cycle was started at 15 hours, 31 hours, and 61 hours of age to demonstrate the appearance of eosinophils in the inflammatory exudate with increasing age. In each instance the sample is taken four hours after the inflammation was initiated. (*a*) Started at 15 hours of age showing 95% polymorphonuclear granulocytes and 2.9% eosinophils; (*b*) started at 31 hours of age and showing 90% polymorphonuclear granulocytes and 5.2% eosinophils; (*c*) started at 61 hours of age and showing 51.4% polymorphonuclear granulocytes and 45.6% eosinophils.

entirely of neutrophils. The observation started at 31 hours of age showed a slight eosinophilia at four hours, while the study initiated at 61 hours of age provided an exudate containing 45% eosinophils.

Because it seemed possible that the observed inflammatory eosinophilia in the infants could be simply a reflection of a high level of circulating eosinophils, differential counts of blood smears and total eosinophil counts were taken at the time studies of the inflammatory cycle were initiated. The correlation graph shown in Figure 8 describes the results of these observations. The infants with the highest circulating levels of eosinophils often had a higher percentage of eosinophils in the exudates, but in every instance the exudate eosinophilia was out of proportion to the circulating levels in the group 2 to 21 days of age. In contrast, two children, 11 years and 23 months of age who had 20% and 13% of eosinophils, respectively, in their peripheral blood, had less than 1% eosinophils in their inflammatory exudate.

The other consistent difference observed in mature and immature subjects studied by this technique was the less rapid and apparently less extensive shift of the exudate cell population from predominantly granulo-

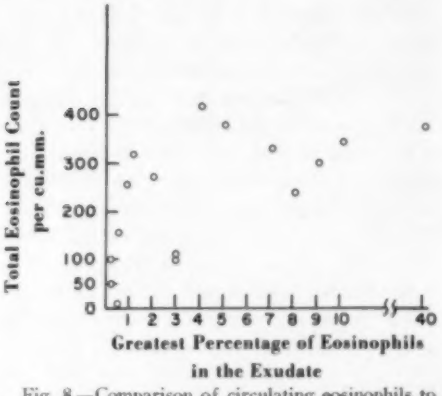


Fig. 8.—Comparison of circulating eosinophils to the eosinophilia in the inflammatory exudate. Total eosinophil counts were done on some of the infants and children at the time of initiation of the inflammatory cycle. While there is a tendency for the more intense inflammatory eosinophilia to be found in the children with the higher circulating eosinophil counts, the total correlation is poor.

cytic cells to mononuclear cells, a difference most marked in the youngest infants studied. Whereas this shift had occurred in mature subjects by six hours, it was inconsistently present in the infants at this time, and, when present, even at eight hours, the proportion of mononuclear cells was usually less than in the older group. This observation is more clearly seen by the differences in mean values for total granulocytes shown in Figure 6, which presents a summary of the data indicated in the scatter diagrams.

Comment

Perhaps, the most significant result of these experiments lies in the general observations that neonatal infants do have the capacity to marshal an early nonspecific inflammatory response remarkably well and that the differences which appear to exist in neonatal and more mature subjects are not major ones. While it may be said, in this general way, that the apparent defect in the local response to inflammatory stimuli in the neonatal period does not lie in any gross deficiency in the nonspecific inflammatory cycle, the qualitative differences which were found could reflect defects in neonatal infants of considerable physiologic importance.

It is essential therefore to examine carefully the limitations of the technique employed and to evaluate the reliability of the qualitative information which has been presented. It is evident that no quantitative information can be obtained by the technique used in this way. The inflammatory exudate is assumed to accrue by diapedesis of cells through injured and uninjured blood vessels in the area subtending the abrasion. Therefore, the sequence of changes in the inflammatory cells would appear to be related to the greater ease with which the polymorphonuclear cell can pass through capillary walls. Accordingly, the later appearance of mononuclear cells would be related to their less rapid rate of transmigration.[9] Evaluation of the technique depends upon the assumption that the various cells appearing on the abraded surface will stick to the overlying glass cover with the same facility irrespective of the morphological characteristics of the cell involved. Certain observations, such as those of Gluck and Silverman [10] indicate that immature leukocytes may have a decreased capacity for effective phagocytosis and a decreased motility when studied in their own plasma,[11] and it would therefore be possible that their tendency to stick to glass might be a related function and similarly impaired. If this were true, certain components of the inflammatory exudate in the very young infant might not stick to the glass as well as others and would fail to be represented in relative counts. As will be pointed out below, it is not thought that such considerations apply to the occurrence of inflammatory eosinophilia in the 2- to 21-day group of infants. However, until further data are available, the relative changes in the proportions of neutrophils and mononuclear cells with time in the young infant as compared with the mature person remain open to this theoretical objection.

The most interesting finding in these studies is the unique inflammatory eosinophilia which occurred in the infants of age 24 hours to 21 days. This observation was made not only in isolated subjects during this period of time but was observed to appear after 24 hours in repeated serial observations of the same infants. It was conceivable that the inflammatory eosinophilia merely reflected a high level of circulating eosinophils in the peripheral blood, the sampling representing simply an aliquot of peripheral blood. However, the failure of inflammatory exudates in mature subjects with known high circulating levels of eosinophils to exhibit eosinophilia and the lack of proportional representation of circulating eosinophils in the inflammatory exudates of newborns render this extremely unlikely. These data strengthen the conclusion that inflammatory eosinophilia in the neonatal period may represent selective accumulation of this cell type in the inflammatory exudate, greater in quantity than can be explained by the numbers in the circulating blood. Evi-

dence bearing upon this conclusion may be found in the studies of Harris and Schick,[12] Mayerhofer and Lypolt-Krajnović,[13] and Matheson et al.,[14] showing that eosinophils are abundant (in our own observations, frequently the only cell type) in the lesions of erythema neonatorum in otherwise normal infants after the first day of life.

Whereas slightly elevated levels of eosinophils are known to be present in such neonatal infants,[15,16] the proportion does not approach that found in the lesions. For example, Matheson et al.[14] found nasal and occasional rectal eosinophilia in normal neonatal infants in whom peripheral eosinophil counts were both high and low; however, a general correlation of mean values existed between the two parameters. The peak incidence of normal nasal eosinophilia in their study was at 3 weeks of age, whereas the highest circulating levels were found between 6 and 7 weeks of age. The incidence of eosinophils or their precursors in the infants' bone marrow was found to have no relation to either tissue or circulating levels. These studies also tend to support the concept of selective accummulation of eosinophils in the tissues of newborn infants.

The functional significance of inflammatory eosinophilia during this period of life is not clear. The well-established association of inflammatory eosinophilia with tissue reactions in which immunological specificity is involved [17,18] has led to speculation on the relationship between erythema neonatorum and the presence of antibody components passively transferred from the mother,[12] and the suggestion of an analogy with serum sickness. Since the established role of eosinophils in immune phenomena is as yet one of "guilt by association" and temporal correlation,[18] the basic premise of this analogy is uncertain. Further, this study indicates clearly that eosinophils accumulate in the exudates of normal infants after entirely nonspecific stimuli.

Another line of thinking which might be applied in attempts to explain the striking inflammatory eosinophilia would depend upon the relationship between adrenal function and the levels of circulating steroids in newborn infants. However, the pronounced discrepancy in the levels of previously measured circulating steroids, particularly after 7 days of age,[19,20] and those which would be expected from the eosinophil counts during the first month of life has failed to provide further stimulus to this approach.

Until the function of the eosinophil is known, particularly in its relation to specific immunological phenomena, it is doubtful that its role in erythema neonatorum and in the inflammatory cycle of neonatal infants can be accurately assessed. The newborn infant is said to be handicapped in his capacity to initiate or complete a classical specific immune response with the production of circulating antibodies. If this concept is valid the appearance of tissue eosinophilia in this period of life offers suggestive evidence against the active participation of the eosinophil in classical immune phenomena. Further studies of both neonatal antibody-forming capacity and tissue eosinophilia are under way at present.

Summary

The inflammatory cycle was studied in newborn infants, older children, and adults, with use of a sterile abrasion as the inflammatory stimulus and with samples of the inflammatory exudate taken at one- to two-hour intervals up to 12 hours. The technique used brought out two qualitative differences between infants and older children or adults: (1) an inflammatory eosinophilia, averaging 19% of the exudate cells, was seen in infants 2 to 21 days of age two hours after the initiation of the inflammation, which was not seen in the infants less than 24 hours of age or in the older children or adults; (2) the shift from the early granulocyte preponderance to a mononuclear cell majority was slower and less intense in the infants studied. With the exception of these two differences the capacity of the neonate to marshall an inflammatory response is morphologically similar to that of the mature subject.

These studies, while failing to provide a clarification of the decreased nonspecific reactive capacity observed in neonatal infants, offer several new lines of approach to the problem.

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Skin-Test Prediction as a Teaching Aid

A Preliminary Report of Test Predictability in Medical Students

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Although undergraduate instruction in allergy has been relatively neglected in many medical schools [1] despite the frequency of allergic disease, there has been a trend towards an increase in the time devoted to this subject in the medical curriculum in recent years. This trend demands a search for new and better pedagogic methods in allergy teaching. The use of medical students as subjects for both demonstration and practice of diagnostic and therapeutic procedures has long been common usage in medical education, and in certain allergy clinics students learn and perfect their technique of skin testing in and upon the epidermis of their peers.

In our experience this teaching exercise had met with but variable enthusiasm. We have therefore attempted during the past five years to add to it an element of dramatic suspense and ego-involvement by venturing to predict the results of the forthcoming tests for each individual student. Such predictions have been based on the hypothesis that definite present, past, or family histories of allergy suggest a high probability for positive skin-test reactions, though there is little in the literature on the feasibility of predicting skin-test results on this or any other hypothesis.

Many standard textbooks on allergy give the impression that allergic persons almost uniformly give positive skin tests, though significant exceptions have been emphasized by Peshkin,[2] Glaser,[3] and others. There is but scant documentation on the prevalence of positive skin-test reactions in "mixed" or "normal" populations; little representing recent contributions on this subject to our knowledge.

In an unspecified number of normal children (25 to 75) of varying ages, Baker [4] (1922) found only 10 positive reactions in 937 scratch tests with food extracts. Peshkin and Rost [5] (1922) found 10% "doubtful" reactions among 502 carefully studied normal children tested by scratch to foods and environmentals. Moro [6] (1932) found 326 normal infants scratch-test-negative to egg white, while Hill [7] (1937), testing intradermally with house dust and goose feathers, found 20 normal infants under one year of age entirely negative. Smyth and Bain [8] (1931) found 125 newborn scratch-test-negative to egg, wheat, and milk. Their family histories in relation to allergy are not stated.

In adults, on the other hand, most workers have found higher proportions of positive reactions among normal persons. Berger [9] (1930) tested 207 "healthy" adults intradermally and found that 56% gave some reaction, 36% gave moderately strong reactions to between one and three antigens, 10% gave strong reactions to four or more antigens, 7% gave very strong reactions to one antigen, and 3% gave very strong reactions to several antigens. Salén [10] (1935) intradermally tested 432 normal bakers and cavalrymen to wheat and horse dander. Thirty-eight per cent of bakers responded positively to wheat and 23% of cavalrymen to horse dander, with no positive reactions among bakers to horse dander or among cavalrymen to wheat. Rackeman and Simon [11] (1935) found 50% of 60 hospitalized patients intradermally positive to rag-

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weed, horse serum, mixed feathers, or cat hair. In retrospect, 10% were shown to have allergic histories. Grow and Herman [12] (1936) tested 150 carefully screened nonallergic adults with egg white, feathers, and horse dander, finding that 55% gave one or more positive reactions.

Pearson [13] (1937), in a careful quantitative study, showed that the frequency of positive reactions was related to the dose of antigen and that in the intradermal doses usually employed in practice 66% of asthmatics, 26% of asymptomatic subjects with positive familial histories of allergy, and 4% of normal adults between 15 and 30 years of age, representing 40, 34, and 50 patients, respectively, gave positive tests to house dust. Fewer positive reactions to chicken feathers, wheat, and egg white in similar dosage were found in all three groups. Data are given for various age groups and antigen dosage, and the reader is referred to this paper for full details.

Ehrstrom [14] (1951) studied a small self-contained population in North Greenland and found that 20 persons of 39 having no allergic manifestations were completely negative to intradermal testing with dog hair, dust, pollens, meat, fish, flour, feathers of Greenland birds, and cosmetics. Nineteen of these persons showed one or more positive reactions, but only two cases gave more than three positive tests. There were 11 positive tests to dog hair and less than half that number to any other single test extract. Rimington and Maunsell [15] (1950) tested 410 patients suffering from "perennial or seasonal rhinorrhea, with or without bronchial asthma, neurodermatitis, or urticaria." Using 0.04 ml. doses of various dilutions of his "standard crude dust antigen" intradermally, he found 267 of these patients "sensitive" to dust. The threshold of skin reactivity in atopics varied from dilutions of the antigen of 10^{-5} to 10^{-10}. "When the patients were divided into allergic and nonallergic groups on the basis of whether or not they had family or personal histories of allergy," 4% of nonallergics gave weak positive reactions with dust antigen in a dilution of 10^{-5}. With use of higher concentrations of antigen the number of reactions in nonallergics increased to 35%. Information on the number of "normals" versus "allergics" and ages are not given.

Herxheimer [16] (1954), in a study of skin tests in asthmatics, tested 300 patients aged 5 to 75 years and 100 normals whose ages are not stated. Puncture tests were performed with grasses; trees; weeds; horse, dog, and cat hairs; house dust; sage; orris root; molds, and bacteria extracts. Of the 100 normals, 49% were negative and 34% gave positive immediate reactions, predominantly to pollens (44%) and to the epidermals (17%). Of the 300 patients, 84% gave immediate positive reactions and only 5% were totally negative.

In a critical review of the subject of skin-test reactions in normal persons, Hill [17] (1940) was unable to advance any more definite conclusion that the generalization that positive reactions appear to be rare in children and not uncommon in adults.

Materials

Consistent records of our experience with the predictability of skin tests were not maintained until early in 1957, at which time we began the collection of data in a systematic fashion, both current and retrograde. In the latter instance, questionnaires approximating our original interrogations were sent to the members of the current senior class who had attended the pediatric allergy clinic the year before and to those members of the junior class who had attended in the fall of 1956. Retesting was offered to those who might have lost their test records, and 20 retests were performed. Current records noting symptoms, past history, family history, prediction, and test results were then maintained on those students who attended allergy clinic from early 1957 to October of that year. Approximately 15 cases in which the questionnaire response or record of testing was ambiguous or inadequate have been excluded from the present report. These were excluded a priori by one of us (E. M. H.) before consultation for prediction with the other (M. Z.), who has acted throughout as the sole predictor of test results.

Sixty-one cases are reported, based on 29 current and 32 retrograde reports. The current reports comprise all students attending allergy clinic from January to October, 1957. These cases include 61

adults, aged 22 to 36 years, with a mean age of 25. The group consists predominantly of white men, though six were women (10%) and three were Asian (5%).

The test extracts used were obtained commercially * and were those used routinely in our clinic. They include 20 seasonal pollens, 15 epidermals and other inhalants, and 45 foods. See Zall [28] (1955) for details.

Methods

University of California at Los Angeles junior students spend four or five half-days of their nine-week combined pediatrics and child psychiatry outpatient clerkship in the pediatric allergy clinic. The first hour of each of these sessions is devoted to a didactic lecture. In the first of these, after an introduction to the nosology and semantics of allergy, the subject of the allergy case history is presented. Toward the end of the first didactic session a brief but salient history is taken from each of the four to six students by the instructor in the presence of the entire group. After this, a gross quantitative prediction of each student's test result is then made by the instructor. Reactions are graded as per Table 1. Each student is predicted to be a negative, doubtful, mild, or strong skin-reactor according to the number of positive tests. Tests are accepted as positive which show significant degrees of erythema and whealing in relation to the basal reactivity of the subject's skin. On rare occasions the reactivity of the student has been up-graded in view of massive reactions to fewer tests than those called for the assigned grade in Table 1. The classification "doubtful" has been predicted as infrequently as possible to avoid any suggestion of willful chicanery.

TABLE 1.—*Grades of Skin-Test Reaction*

Positive Tests, No.	Test Grade	Patient Designation
None	Negative (—)	Nonreactor (—)
0-2	Doubtful (±)	Doubtful (±)
3-8	Mild positive (+)	Mild reactor (+)
9 or more	Stronge positive (++)	Stronge reactor (++)

During the second didactic session, one week later, there is an exposition of the theory, limitations, and techniques of skin testing. After a word about the management of adverse reactions, the students proceed to their mutual scratch testing. Intradermal tests are not performed until the following week. All test results are read and interpreted by the instructor in the presence of the entire group and recorded by the students. When all testing is completed, results are compared with predictions. Unfortunately, time and availability of equipment and materials has not always permitted complete testing with the 80 antigens usually employed in patient-testing in our clinic. Of the 61 cases reported, 25 had complete testing (80 scratch and 40 intradermal tests), 30 had half complete testing or more (40 scratch and 20 intradermal), while 7 had testing with less than this number of antigens. With the exception of cow's milk casein, cow's milk lactalbumin, goat milk, egg white, and wheat, food antigens have been used only for scratch testing. When moderate to strong positive reactions occurred by scratch test to any given antigen, intradermal testing with that antigen was not performed. Though the initial choice of testing materials was not infrequently conditioned by the history obtained, further testing was never undertaken in an attempt to reconcile discrepancies between test and prediction.

TABLE 2.—*Grading of Correlation of Test to Prediction*

Test Result	Prediction			
	Negative	Doubtful	Mild	Strong
Negative	+	±	—	—
Doubtful	±	+	±	—
Mild	—	±	+	±
Strong	—	±	+	+

Table 2 shows our scheme for the classification of degrees of correlation between test results and prediction. Of 16 possibilities, 5 each are designated positive and negative, providing equal weighting. Six of the sixteen are taken as doubtful, giving that group a slight statistical advantage. Negative correlations represent those predictions wrong in nature or of significantly wrong degree, doubtful correlations those not quite right but not wholly wrong, and positive correlations those where the prediction closely matched the test result.

With pollen antigens 1,060 scratch and 920 intradermal tests were performed, yielding 62 and 79 positive reactions, respectively. Of 980 scratch and 900 intradermal tests with inhalants and epidermals, there were 9 positives in the former and 49 in the latter. Of 1,340 scratch tests to food antigens, 26 gave positive reactions.

Results

Figure 1 shows our data on the atopic group (symptomatic). Of 28 cases, 6 had

* Hollister-Stier Laboratories.

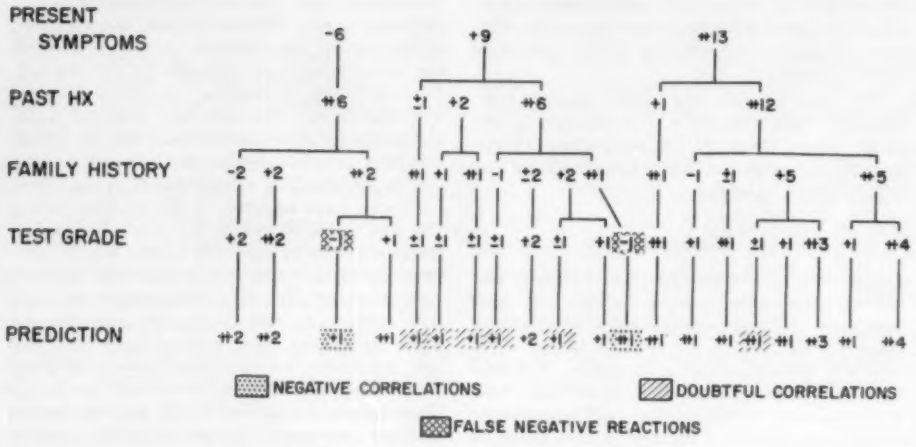

Fig. 1.—Data from atopic (symptomatic) group (28 cases).

no current symptoms but gave strongly positive past histories, 2 of asthma and hay fever, 3 of hay fever alone, and 1 of allergic rhinitis. Twenty-two were having manifest symptoms, including three cases of asthma, nine of hay fever, eight of allergic rhinitis, and one of severe urticaria. These 28 represent 46% of the series.

Of these 28 atopic subjects, 60% gave a significantly positive family history. This is in contrast to the nonatopic group, where only 24% had family histories of allergy. Twenty students (71%) showed mild-to-strong skin reactions, six had doubtful degrees of reaction, and two were nonreactors. These latter we have classified as "false" (that is to say, unaccountably) negative reactors. As is shown, both of these had strongly positive past and family histories and one had current symptoms. Their predictions were both 1+, and they comprise the two negative correlations in the atopic group. Six of nine cases showing only doubtful reactivity had been predicted 1+. These comprise all the doubtful correlations of the group. In retrospect, it was noted

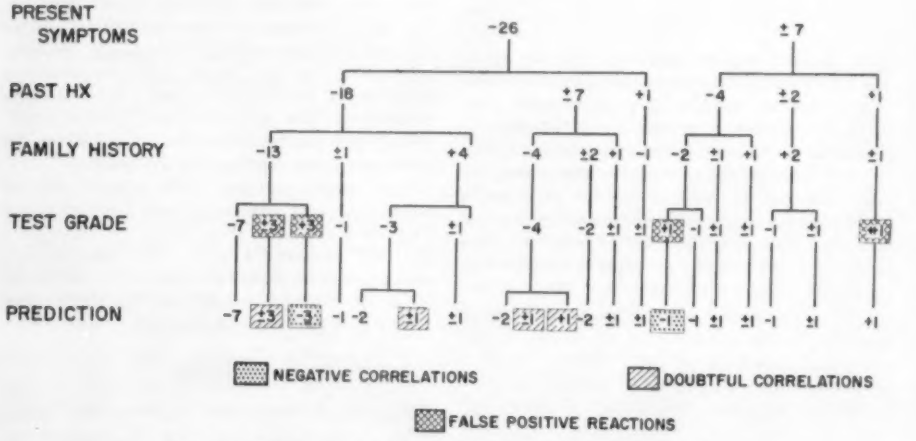

Fig. 2.—Data from nonatopic (asymptomatic) group (33 cases).

that all were having mild symptoms of a nonseasonal nature to which we now attach prognostic significance. In this regard, it is noteworthy that the three cases with positive skin tests in the mild symptom subgroup all have seasonal components.

In Figure 2 we see data on the nonatopic group (asymptomatic), of which 26 subjects (79%) had no allergic symptoms and 7 had very questionably atopic complaints. Only 7 students (24%), as previously noted, gave positive family histories. Nineteen students (57.5%) had completely negative skin-test reactions. Nine students (27%) were doubtful reactors, and five students (15%) had positive skin tests. The five reactors included all four negative correlations in the group, and these, along with three doubtful reactions in cases having no element of atopy by history, have been designated as the eight "false" positive reactions. The remaining three doubtful correlations show no common element save an overevaluation of one or another anamnestic element when the prediction was made.

A history of drug sensitivity, primarily to antibiotics, was obtained in seven nonatopics. Six of these were negative skin-reactors, while the seventh showed but doubtful reaction. Drug sensitivity was not a useful clue to positive skin tests, as has been noted on many occasions in the literature.

Table 3 shows our over-all degree of predictability. Predictions were positive in 71% of atopics and 69% of normals, doubtful in 21% of atopics and 18% of normals, negative in 7% of atopics and 12% of normals. If negative results alone are considered as being "completely wrong" predictions, in atopy our predictions are 92% reasonably correct and in nonatopics the figure is 88%.

TABLE 3.—*Skin-Test Predictability*

Correlation of Test with Result	Group	
	Atopic (28)	Nonatopic (33)
Positive	20 (71.4%)	22 (69.7%)
Doubtful	6 (21.4%)	6 (18.1%)
Negative	2 (7.1%)	4 (12.2%)

Comment

The results reported here on skin-test predictability are, of course, open to the criticism of subjectivity. That is to say, the skin test results, being read by their predictor, might well be interpreted in a subjective manner, so as to raise the number of positive correlations between result and prediction. As implied in the introductory remarks, this technique evolved over a period of time and was not documented until late in its use. Grading of tests results by an independent observer unaware of the history or prediction would unquestionably be preferable. A mitigating factor against excessive subjectivity, however, has been the presence of the students throughout the procedure. Their critical attitude and not infrequent scepticism has in no small part imposed an element of objectivity on the test readings and gradings of predictability.

A potential objection is also inherent in the variability of degree of testing, i. e., number of tests applied in each case. A review of the data, however, shows no statistically significant difference in the degree of testing among various categories of prediction or outcome, and thus this uncontrolled variable does not appear to have skewed the data.

The population covered in this report would appear to show a higher percentage of allergy than has been noted in the general population. Though this group is unselected as to the prevalence of allergic disease by any medical screening, it is highly selected as to I. Q., socioeconomic status, educational level, etc. A recent study of asthma among undergraduates at the University of Wales by Grant [19] shows that the frequency of this allergic disease is higher among these university students than in the general population of the area from which they derive. The author comments on some of the compensatory factors which

might tend to concentrate allergic persons in fields of intellectual pursuit, and these may well be operative to produce the relatively high incidence of allergy recorded in this series.

It must be emphasized that only the gratifying degree of medical sophistication demonstrated by the junior medical students permits of sufficient degree of accuracy to warrant prediction of skin-test results, especially in view of the minimal history that time permits us to obtain. Similar practices with patients are not to be condoned, as is repeatedly reiterated to the students. Substance-specific predictions should not be attempted and group-specific predictions, but rarely.

Throughout we have used the terms allergy and atopy interchangeably. By either we refer in this context to that hereditarily conditioned human immunologic state of hypersensitivity in which minimally antigenic materials, absorbed by natural routes, stimulate the production of (*a*) a type of antibody, "reagin," demonstrable in vivo by passive transfer techniques; (*b*) positive immediate cutaneous reactions of the wheal and flare variety to tests with extracts containing the specific antigens, and (*c*) the clinical syndromes of asthma; atopic dermatitis; allergic rhinitis, including hay fever, and urticaria.

The levels of skin-test predictability observed in this study in medical students appear amply adequate for their use in teaching. They call for neither apology nor retraction on the part of the instructor. On the contrary, they tend to affirm the solid basis of clinical allergy and to facilitate and enliven student-instructor rapport. The incidence of negative and doubtful correlations serves well to illustrate the limitations of skin testing, even in experienced hands. The procedure described has been received with enthusiasm by the students and has provided many opportunities for the confrontation of conflicting opinion, the clarification of the didactic material under consideration, and the emphasis of basic principles in allergy. Searching questions which might otherwise have gone unvoiced have often been directly provoked by the use of such test predictions.

Conclusions

The literature relating to the correlation of history with the results of skin testing gives no clear impression as to the percentage of "nonallergic" persons of various ages who react positively to skin testing. In young adult medical students we find that a history of clinical allergy, personal or familial, will usually forecast positive skin-test reactions. On the other hand, if the average medical student cannot recall any allergic symptomatology or knows of no familial history of allergy, negative skin tests are likely. The ability of the instructor in pediatric allergy to demonstrate the relative predictability of skin testing in medical students is an adjunct to the successful teaching of this subject.

Summary

Sixty-one medical students, 28 atopic and 33 nonatopic, were examined for predictability of skin-test results.

Precise predictability was found in 71% of the atopic group and in 69% of the nonatopic group.

"False positive" reactions are three times as frequent as "false negative" reactions and will occur in about one-fifth of "nonatopic" persons.

The prediction of skin-test results in medical students is a useful teaching method.

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Case Reports

Atropine Poisoning with Psychosis

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The subject of atropinism has been discussed previously in case reports, but all of these reports have had something a little different to contribute. Atropine intoxication or poisoning is not infrequent, but cases in which the mental symptoms dominate the picture are quite rare. The case reported below is an illustration of the mental symptoms predominating. The child was studied and treated as an outpatient by the Psychiatric Service of the University of Alabama Medical School.

A large majority of these cases can be accounted for by therapeutic accidents which result from carelessness on the part of physicians, pharmacists, patients, or unskilled hospital aides. There have been reports in the literature which are examples of this fact. Our case does not fit into this category but is thought rather to involve a person who had increased susceptibility or perhaps idiosyncrasy to this drug.

Atropine intoxication occurs rather frequently when the drug is administered either intramuscularly or subcutaneously or when large doses of atropine (as found in poisonous berries, for example) are ingested by mistake. However, intoxication is infrequent when atropine is used in solution or in an ointment applied to the eyes or nose. There are several cases in the medical literature in which the typical symptoms of poisoning are described as fever, malaise, reddening of the face, dryness of the mouth, accelerated pulse rate, and nausea.

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These symptoms are most frequently seen. The cases with mental symptoms are really very rare and will be cited in the following paragraphs.

Several cases which have been reported previously will be mentioned here briefly, since they have some similarity to the case which we observed:

(1) Métivier's case [1] of a 74-year-old woman showing acute confusion, excitement, and hallucinations after the instillation of 1% atropine eye drops.

(2) Duggan's case [2] of a 10-year-old boy who developed acute confusion after the instillation of 1% atropine drops.

(3) Hopkin's case [3] of a 10-year-old boy who became confused, violent, and delirious after instillation of 1% atropine eye drops.

(4) C. Alston Hughes' case [4] of a 9½-year-old girl who, after 1% atropine eye drops, developed fever and skin changes and became delirious at night.

(5) Morton's case [5] of a 3-year-old child who became restless and nauseated and developed gastrointestinal symptoms after receiving 1% atropine solution in the eyes.

(6) Belz's case [6] of a 10-year-old girl who developed a psychosis after instillation of 1% atropine in the eyes.

(7) Heath's case [7] of a 2-year 9-month-old boy who had 1% atropine ointment applied to his eyes twice a day for two days. On the third day the child had a seizure (tonic-clonic) and became semiconscious. The pulse and respiration were accelerated, petechiae formed, pupils were dilated and unresponsive to light, and he developed slight opisthotonos. Cerebrospinal fluid was normal. The boy died four days later.

The toxicity of atropine will be mentioned briefly. The usual dosage of atropine ap-

plied to the eyes is one or two drops of a 1% solution (this corresponds to a $\frac{1}{120}$ of the alkaloid). This amount seldom causes any toxic symptoms unless a special susceptibility is present. When the toxic symptoms occur they are mostly attributable to parasympathetic paralysis and are very rarely characterized by mental disturbances. The toxic symptoms as they are related to dosage are as follows: $\frac{1}{30}$ of a grain causes parasympathetic paralysis; $\frac{1}{12}$ of a grain causes restlessness; $\frac{1}{6}$ of a grain causes ataxia and mental symptomatology.

Fortunately, the mortality rates are low, probably because of the rapid excretion and inactivation of the drug.

In cases of atropine intoxication where mental symptoms have been observed, the commonest manifestations have been confusional states, vivid visual hallucinations, restlessness, muscular incoordination, and increased emotionalism. Sometimes the symptoms take the form of a schizophrenic episode. Differential diagnoses to be considered are schizophrenia, manic-depressive disease, and other intoxications.

In our patient, as well as in others reported who have had systemic symptoms as a result of atropine eye drops, the absorption into the blood stream through the mucosa of the nasolacrimal duct was rather rapid. The most rapid absorption of atropine occurs when it is given subcutaneously, and the slowest is when it is given orally. In terms of rapidity, the absorption that takes place through the eye and lacrimal apparatus is intermediate between these two routes.

Report of a Case

A 10-year-old Negro boy was referred to the Ophthalmology Clinic of the University of Alabama Hospital on Oct. 11, 1957 at the request of his schoolteacher, who thought that he had a visual disturbance. When he was seen at the eye clinic, a diagnosis of alternating esotropia was made and 1% atropine eye drops were prescribed. The dosage was two drops in each eye three times a day. Two days later his foster mother brought him to the emergency room of the University Hospital. At 2 a. m. on Oct. 13, 1957, the pediatric resident, who had examined the child in the emergency room, called the psychiatric resident into consultation because of the boy's bizarre behavior.

He was a well-developed well-nourished child who was quite agitated and uncooperative. Temperature was normal, pulse 120, and mucous membranes dry. Pupils were widely dilated and did not react to light. It was difficult to examine the fundi, but the retinae seemed to have a generalized hazy appearance. The heart and lungs were entirely normal. Tendon reflexes were hyperactive.

The history as it was obtained from the foster mother revealed the following facts: The previous night she had given him his eye drops and put him to bed at 10 p. m., and soon after this she heard noises from his room. She found that the boy was acting as though he were drunk. He had apparently fallen out of bed and cut his lip, but when she saw him he was talking to himself in a babbling fashion, staggering and running about. While doing all this, he was also tearing up objects and picking up rugs.

Further history at the time of first mental examination revealed that this child had always been mentally defective, had eye trouble, and had a speech impediment. His real mother had become mentally ill after his birth and had been committed to the State Mental Hospital where she subsequently died.

The child is a twin, the younger of the set. The foster mother denied that the patient had ever manifested this behavior previously. The twin was said to be relatively healthy.

A provisional diagnosis of an acute psychotic reaction to atropine was made, and the family was told to discontinue the atropine drops. Chlorpromazine (Thorazine) was prescribed at a dosage of 25 mg. 3 times a day in an attempt to control the behavior, and the child was sent home with instructions to return the next day. At that visit (12 hours after his last eye drops had been given) he was slightly improved. He was not agitated and not really uncooperative. The chlorpromazine was continued, and the child was sent home, to be seen the next morning. On the morning of Oct. 14, 1957, the child was much improved. Stanford-Binet and TAT Tests showed that he had definite mental retardation (I. Q. of 60 and a mental age of 6 years), and his behavior on the personality tests revealed perseveration, autistic thinking, defective reality-testing, and bizarre thought content. Hallucinations and delusions were also suggested from the test results. Reflexes were still hyperactive.

His third clinic visit was on Oct. 17, 1957, and at this time the child's behavior was almost normal for him. The pupils were still dilated and reacted sluggishly to light. The foster mother was told to discontinue the chlorpromazine and that the child could be returned to school.

The last visit was on Nov. 8, 1957. At that time,

he was getting along very well, was quite sociable, and answered questions appropriately for his mental age. His foster mother reported that he had been doing very well indeed. The patient at this time was able to recall certain parts of his psychotic episode.

Physical examination at the time revealed nothing outstanding. Psychological examinations showed none of the psychotic signs mentioned before but rather revealed that his mental deficiency seemed to be the primary determinant of his behavior at this time. The tests showed evidences of overdependence, vague fears, and aggressive impulses. His capacity for reality testing was thought to be limited, and his present adjustment was considered borderline.

Comment

It is remarkable that this child had a very small amount of atropine (a total of eight drops in each eye) and yet he developed a psychotic reaction with visual hallucinations, hyperexcitability, behavioral disturbance, and personality changes which were manifested in the psychological tests (perseverance of one idea, autism, delusions, hallucinations). The patient did not have fever, gastrointestinal disturbances, or some of the other signs of atropinism.

The neurological findings were enlarged fixed pupils and deep tendon hyperreflexia, but there were no other significant physical findings. We first considered two possibilities: (1) a superimposed schizophrenia in a retarded birth-injured child; (2) an acute intoxication due to the atropine drops. Since the patient had such a small dose of atropine, we questioned the diagnosis of intoxication and sent the child home to be followed up at the Psychiatric Clinic. The absence of mental signs after 22 days, tested by both psychiatric and psychological examinations, proved to our satisfaction that the condition was an acute intoxication of atropine.

One should keep in mind that atropine is one of the alkaloids to which individual susceptibility is variable. Caution in the administration of the drug is desirable.

Our treatment differed from that usually used in these cases. Morphine and pilocarpine are customarily used, although there is no treatment that is actually specific in these cases. The unusual feature of our case was the predominance of mental symptoms and the complete absence of the more usual manifestations of atropine poisoning.

Miss Carolyn Calhoun, Staff Psychologist, Department of Psychiatry, University of Alabama Medical College, administered and interpreted the psychological tests used in this study.

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Phenylketonuria Treated from Earliest Infancy

Report of Three Cases

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It has been suggested that an assessment of the effects of the dietary treatment of phenylketonuria might best be made by observing the developmental pattern of affected children treated from the neonatal period.[1] The purpose of this paper is to report the developmental patterns of three patients, aged 3½ years, 2⅓ years, and 1 year, respectively, who have received a phenylalanine-deficient diet continuously since early infancy.* The dietary program was one that has been outlined in detail by Armstrong and Tyler.[5] A phenylalanine-free diet consisting of a commercial protein hydrolysate with fat and carbohydrate supplements was given for three weeks. On such a diet the serum phenylalanine level returned from abnormally high to normal levels. After the initial three-week period, 2 gm. of natural protein in the form of cow's milk (2 oz.) were added to the diet. At about 6 months of age, the natural protein was increased to 3 gm. a day. On such a diet, the serum phenylalanine level was maintained between 3 and 7 mg/100 ml.

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Marvin D. Armstrong, Ph.D., E. C. Binkley, Jr., M.D. and Linda Hassel, Ph.D., collaborated in this study.

From the Department of Pediatrics and Division of Neurology, University of Colorado School of Medicine.

The project was supported by a grant from Mead Johnson & Company, Evansville, Ind. The phenylalanine-deficient diet was supplied by Mead Johnson & Company (Lofenalac) and by Merck & Co. Inc., Rahway, N. J. (Ketonil).

The early development of the two oldest infants (Cases 1 and 2) was previously reported,[2,3] while the metabolic changes in the neonatal period of the youngest patient have been studied in detail and previously reported by Armstrong and Binkley.[4]

Report of Cases

CASE 1.—A boy, born Feb. 4, 1955, sibling of a patient with phenylketonuria (Case 2, Horner and Streamer[2]), developed a positive urinary ferric chloride test by 7 weeks of age, subsequent to negative urinary ferric chloride tests in the newborn period and at 5 weeks of age. The serum phenylalanine level at 7 weeks of age was 55 mg. %. At this time a phenylalanine-deficient diet was started.

TABLE 1.—*Physical Growth of Patient 1*

Age	Head Circumference, In.	Height, In.	Weight, Lb.-Oz.
Birth	13½	19½	7-7
7 wk.	15½	22	11-5
* 6 mo.	17	25½	15-9
1 yr.	17½	27½	18-8
2 yr.	18¼	29½	23
3 yr.	19¾	34½	30-8

* Phenylalanine-deficient diet introduced.

The physical growth of this patient is summarized in Table 1.

Motor development has shown a normal progression. Head control was achieved by 3 months. Rolling from the prone to supine position occurred at 4 months. He sat alone at 6 months and walked without aid at 11 months. By 18 months he was running, and shortly afterwards he began climbing and jumping. The development of his intellectual activity has paralleled the motor progress. He vocalized at 3½ months, repeated words at 14 months, and said simple words, such as "hat," "hi," and "yes," by 17 months. At the present time, he speaks in full sentences. Toilet-training was completed by 3 years of age. Four electroencephalograms on the patient have been without abnormalities. When examined most recently (age 3½ years) his pattern of activity was entirely normal.

The motor and mental development in this patient have been quite similar to that of an unaffected brother and stand in marked contrast to the development of an older affected sister, who at 4½

TABLE 2.—*Physical Growth of Patient 2*

Age	Head Circumference, In.	Height, In.	Weight, Lb.-Oz.
Birth	14	18½	5-8
2 mo.	14¼	21½	7-6
4 mo.	15¾	22¾	9
1 yr.	17	28	15-10
2¼ yr.	18½	32½	25

years of age did not speak, was not toilet trained, and was totally unmanageable (Case 2, Horner and Streamer[3]).

CASE 2.—A girl, born April 26, 1956, the sibling of a known phenylketonuric (Case 5, Horner et al.[3]), was found to have a rapidly rising serum phenylalanine level in the first few days of life. The urinary ferric chloride test remained negative until she was 5 weeks old, when the serum phenylalanine level was 65 mg/100 ml.[4] Treatment with a phenylalanine-deficient diet was begun at this time.

The physical growth pattern is shown in Table 2.

The developmental pattern has been satisfactory. She held her head erect at 3 months, sat alone at 7 months, walked alone at 12 months, and ran at 19 months. Vocalization was noted at 2 months. She repeated words at one year and named objects at 14 months. By age 2¼ years, she put two or three words together. Her play activity has always been that of other children her own age. The development of this patient has been in marked contrast to an older affected sister who at age 5 years does not speak, is not toilet-trained, and for whom institutionalization has been arranged.

Although this patient had a convulsive episode after an injection of combined diphtheria and tetanus toxoids and pertussis vaccine (D. P. T.) at 14 months, there have been no abnormalities noted on eight electroencephalograms.

TABLE 3.—*Phenylalanine Levels During First Days of Life of Patient 3*

Day of Life	Serum Phenylalanine, Mg/100 Ml.	Urinary Test	
		$FeCl_3$	DNPH *
0	9 (cord blood)	—	—
1	17	—	—
4	34	—	—
7	--	+ (trace)	+
9	--	—	+
10	75	+	+

* DNPH (dinitrophenylhydrazine) 3 cc. of a saturated solution of dinitrophenylhydrazine in 1 N HCl is added to an equal volume of urine. A yellow turbidity developing within 3-5 minutes indicates a positive reaction for phenylketonuria.

CASE 3.—A girl, born May 23, 1957, is the sister of two severely retarded phenylketonurics.

A sharply rising serum phenylalanine level was noted in the first few days of life as recorded in Table 3.

Treatment with a phenylalanine-deficient diet was started on the 10th day of life. The growth of this infant in the first year of life is shown in Table 4.

In the motor sphere her development has followed a healthy pattern. She held her head erect at three weeks of age, rolled from the prone to supine position at 3 months, and sat alone at 6 months. Three days prior to her first birthday she began walking alone, and on her first birthday she began feeding herself. She now babbles constantly and repeats words.

Two electroencephalograms on this patient have been normal.

TABLE 4.—*Physical Growth of Patient 3*

Age	Head Circumference, In.	Height, In.	Weight, Lb.-Oz.
Birth	13	21	9-5(+)
6 mo.	16½	26½	16-4
1 yr.	17	28¼	19-8

Comment

The developmental pattern in the three infants with phenylketonuria herein reported is considered to be normal. Fourteen electroencephalograms on these three patients have shown no abnormalities. Case 2 has had no further convulsions since the one after immunization with combined diphtheria and tetanus toxoids with pertussis vaccine, and has not received anticonvulsant drugs. Each of these cases has one or more severely retarded siblings with phenylketonuria as well as normal unaffected siblings. The development of each of the above three patients has compared favorably with that of an unaffected sibling and has been in marked contrast to the development of affected siblings.

It has been postulated that the mental deficiency pattern in phenylketonuria is secondary to the elevated serum phenylalanine or its metabolites, and phenylalanine-deficient diets were introduced with the hope that the restoration of the serum phenylalanine to normal would result in an improve-

ment in the mental picture.[1,6] To date, the changes observed in older infants and young children under dietary treatment have been quite variable and, though occasionally quite gratifying, more often have been disappointing.[7-9]

If the mental deficiency in phenylketonuria is secondary to the elevated serum phenylalanine level, the maintenance of this level in the normal range from earliest infancy might be expected to result in normal mental development as reported herein. Since recent reports indicate that some cases of untreated phenylketonuria may have normal or near normal intelligence, one cannot be certain the healthy pattern in these three cases is the result of dietary treatment.[10] However, such a pattern of development in three successive phenylketonuric patients is of considerable interest and suggests that the picture of severe retardation may be prevented by early diagnosis and treatment.

It is of interest that in some cases the presence of phenylketonuria may not be detected by urinary test with $FeCl_3$ or dinitrophenylhydrazine until several weeks of life. In none of our cases was it found before the seventh day of life. Negative urinary tests for phenylketonuria may be misleading before 7 weeks of age. However, the diagnosis may be made before the development of a positive urinary test if a sharply rising serum phenylalanine level is present.

Summary

The developmental patterns of three patients with phenylketonuria treated from the onset of a positive urinary $FeCl_3$ test are reported. After 3½ years, 2¼ years, and 1 year, respectively, the patterns of mental and motor activity are considered to be normal. It is suggested that the typical picture of severe motor and mental retardation may be prevented by early treatment. Comment is made regarding the variability of the time of appearance of positive urinary tests for phenylketonuria and of the value of serum phenylalanine determinations in the neonatal period.

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Incontinentia Pigmenti (Bloch-Sulzberger Syndrome)

A Generalized Disease

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Incontinentia pigmenti is a rare disease. Because it is regarded as a dermatologic problem, few cases have been reported in the pediatric literature. This case is presented to illustrate the multisystem involvement which may be seen in the infant.

Report of Case

A 9-month-old white girl was admitted to Michael Reese Hospital on Feb. 28, 1957. The family history was negative, the mother had not been ill during pregnancy, and the delivery was normal. The history of illness dates from the second day of life, when a generalized vesicular eruption appeared and a diagnosis of epidermolysis bullosa was considered. She was treated with oral hydrocortisone (Cortef) and Mazon (a combination of phenolic substances and organic mercury compound) and Terra-Cortril (an ointment containing 3% oxytetracycline HCl and 1% hydrocortisone, free alcohol) ointments. She was also placed on hypoallergenic soya formula (Sobee) feedings. As the bullae disappeared, a generalized eruption was noted consisting of purpuric, urticarial, papular, and warty characteristics. The child did well until age 5 months. She gained satisfactorily in both height and weight, had good head control, and was able to sit without support.

Her cutaneous lesions aroused interest, and she was presented before a meeting of the Indiana Dermatological Society. The diagnoses suggested were incontinentia pigmenti and pachyonychia congenita.* At age 5 months she developed a temperature of 105 F, a bulging fontanel, and convulsions, and she was hospitalized. Spinal fluid examination revealed pleocytosis, mostly lymphocytes, an elevated protein, and normal glucose. Bacteria and fungi were not found on smear or culture. No fluid was obtained on subdural tap. At this time pox-like lesions were noted on the forehead, and smears of these revealed inclusion bodies when stained with Giemsa's reagent. A serum complement-fixation titre of 1:64 for herpes simplex was obtained at the laboratories of the State Board of Health, Indianapolis. Treatment with chloramphenicol and penicillin was started and continued until an intermediate strength and second strength P.P.D. were found positive. At this time isoniazid (INH) and streptomycin were started and continued for three months. She was then transferred to the Indiana University Medical Center for further evaluation. The diagnosis at the time of transfer was incontinentia pigmenti and possible chronic pachymeningitis due to herpes simplex. Significant findings on this hospital admission were streaked pigmentations located on the trunk and extremities, strabismus, nystagmus, optic atrophy, poor head control, mild opisthotonos, hyperactive tendon reflexes, a wailing cry, and a right hemiparesis. Subdural taps were performed, yielding substantial amounts of bloody fluid from the right side and clear fluid from the left, with a high lymphocyte count. Several lumbar punctures again showed pleocytosis, elevated protein, and negative culture for pyogenic and acid-fast organisms. An electroencephalogram revealed rather marked asymmetry, suggesting involvement of both the left and right hemispheres. A pneumoencephalogram moderate symmetrical ventricular dilatation with diffuse atrophy. The hospital stay was uneventful, and she was discharged after one month.

Eight days later she was admitted to Michael Reese Hospital with fever and a draining ear. At this time she was 9 months old, weighed 13 lb. 8 oz., was 27 in. long, and her head circumference was 41 cm. She could neither lift her head nor sit and did not seem to understand or respond. She exhibited peculiar athetoid-like movements of all extremities, and the neurologic and ocular abnormalities previously found were confirmed. Examination of the skin revealed linear hyperkeratotic lesions (Fig. 1) on the left lower and

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* Dr. Thomas J. Conway of Terra Haute, Ind., and Dr. Charman Palmer, Indiana University Medical Center, Indianapolis, provided detailed reports about this patient.

Fig. 1.—Linear hyperkeratotic lesions, plantar surface, left foot.

left upper extremities, as well as beneath the chin and under the nails (Fig. 2). A diffuse "flowery" type of hyperpigmentation was also present on the trunk and extremities (Fig. 3).

Fig. 2.—Subungual hyperkeratosis, toes, left foot.

Biopsy specimens (Figs. 4 and 5) were compatible with the diagnosis of incontinentia pigmenti.

Spinal fluid was xanthochromic, contained no white cells, and showed normal glucose and protein. Subdural tap showed 10 cc. of clear fluid on the left side with a protein content of 760 mg. %. Pseudomonas aeruginosa was recovered from the aural discharge. Mantoux 1:1,000 and P. P. D. No. 2 were both negative. An EEG was interpreted† as showing multiple foci of spike seizure activity. A pneumoencephalogram revealed dilated

† Dr. Frederick Gibbs, Chicago, interpreted the EEG.

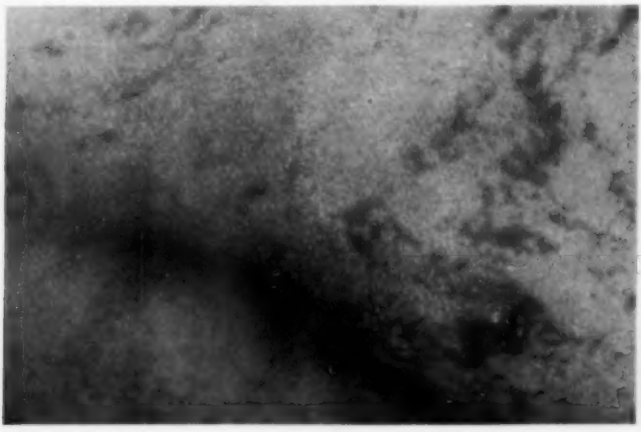


Fig. 3.—"Flowery" type of hyperpigmentation, trunk.

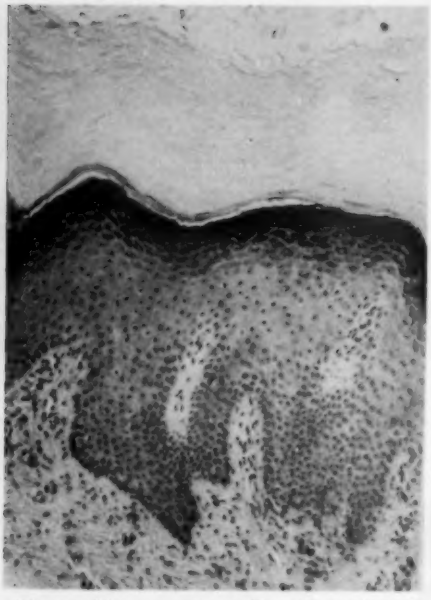


Fig. 4.—Biopsy specimen. Hyperkeratosis, acanthosis, and nonspecific inflammatory infiltrate.

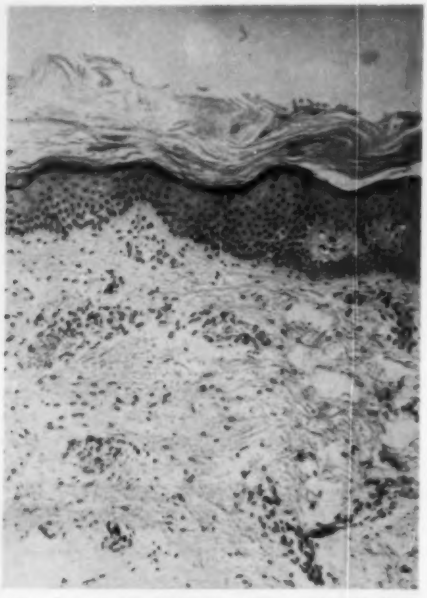
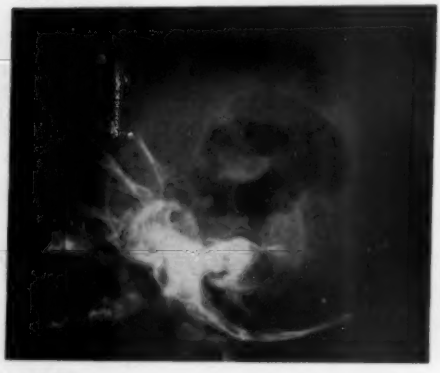


Fig. 5.—Biopsy specimen. Decreased melanin in basal layer and increased melanin in upper dermis.

ventricles (Fig. 6) and a porencephalic cavity in the right parietal region.

The child did satisfactorily in the hospital and with the aid of conscientious nursing took her feedings well. The ear infection disappeared with appropriate antibiotic treatment. She was presented before the Chicago Dermatological Society, and there was general agreement with the diagnosis of incontinentia pigmenti. She was discharged on March 21, 1957, and was not seen until July 19, 1957. The hyperpigmentation was still very obvious, but the keratotic lesions had disappeared.

Fig. 6.—Pneumoencephalogram, dilated ventricles and porencephalic cavity in the right parietal region.



She was able to sit with support and could hold her head well. She had no teeth. She was last seen on Feb. 7, 1958, at the age of 22 months, when she weighed 19 lb. 7 oz. and was 32 in. long. She was still unable to sit without support, and her only developmental advancement was two upper frontal incisor teeth. The skin pigmentation was still present. Blood was drawn on two occasions for repeat herpes simplex complement-fixation test, but because of technical difficulties the procedures were not completed.

Comment

Incontinentia pigmenti (Bloch-Sulzberger syndrome) is a disease characterized by developmental defects of the skin and its accessory structures, ocular and central nervous system defects, osseous deformities, and other mesodermal anomalies.[1] Although this disease is well known by dermatologists, very few descriptions have appeared in the pediatric literature.[2,3] The primary intention of this report is to further acquaint pediatricians with this disease and to emphasize its generalized nature.

The disease was first recognized by Bardach,[4] Bloch,[5] Sulzberger,[6] Naegeli,[7] and

Siemens.[8] The following are the clinical characteristics of the skin in its various stages:

1. The erythema and vesicular stage usually begins shortly after birth and occurs in from 30%-50% of the patients. The lesions are accentuated on the flexor aspects of the extremities and the lateral aspects of the trunk. They persist for several months and then subside.

2. The verrucous stage occurs in about 30% of the patients and slowly subsides after a period of weeks to months.

3. The pigmentary stage begins during the first few years of life and consists of pigmented macules. These are bizarre, irregularly shaped, and irregularly disseminated areas of pigmentation of tan, brown, or slate color. As previously mentioned, the trunk and extremities are the commonest location. The margin of the lesions may be jagged, sharply outlined, or may appear as confluent figures like "veins in marble." Sulzberger[6] mentions that "there is no definite systematization, no segmentary arrangement and no conformity to lines of cleavage, nerves or blood vessels." The pigmentary lesions may occur in areas not involved by inflammatory lesions.

Histochemical studies in various stages of the skin manifestations reveal alterations in tyrosinase activity.[9] Further studies may show this to be present not only in the skin but in other organ systems.

It is considered by some to be a hereditary disturbance[6,7] and by others[10-12] to result from unfavorable intrauterine influences, possibly a virus infection of mother and fetus. Whatever the etiology, a striking characteristic is the multiplicity of organs involved (Table).

Of interest is the complement-fixation titre of 1:64 for herpes simplex discovered at age 5 months. A positive complement-fixation test is related to previous herpetic infection,[18] and while herpes simplex antibodies may be transmitted transplacentally, these usually would not persist beyond age 2-3 months.[19] Also, a smear of the pox-like lesions on that admission revealed inclusion bodies. The acute febrile illness at age 5 months in our patient may have represented a recent herpes simplex infection superimposed on an already existing case of incontinentia pigmenti, or possibly herpes simplex virus had actually affected the infant previously in the fetal stage. Repeat

Defects Reported in Incontinentia Pigment *

		Cases Reported in the Literature						
	Present Case	Carney[1] (37 Cases)	Loveman[2] (3 Cases)	Miller[13] (1 Case)	Epstein[14] (1 Case)	Kitamura[15] (21 Cases)	Cramer[16] (6 Cases)	Philpott[17] (2 Cases)
Delayed or imperfect dentition	x	11	2			3	2	2
Osseous deformities		8						
Alopecia		7	1		1	1	1	
Nail dystrophy	x	3		1			1	
Nervous system								
1. Paralysis	x	7			1			
2. Convulsions	x	4	1	1	1	2	1	
3. Mental deficiency	x	4						
4. Microcephaly	x	4				1		
Eye								
1. Optic atrophy	x	2	1					
2. Strabismus	x	5				1		
3. Corneal opacities		3				1		
4. Cataracts		2				1		
5. Blue sclerae		2						
6. Nystagmus	x	2						
Growth retardation	x	3			1			

* Also reported are single instances of congenital heart disease, retrobulbar glioma, absence of retinal pigment, nevoid patch of the retina, myopia, retrolental fibroplasia, metastatic ophthalmia, urachal cyst, supernumerary ears, sweat disturbance, and hydrocephalus. Parents and siblings may also have developmental defects.

titers might have been helpful in this regard, and it is unfortunate that these were not obtained. However, the close resemblance between this case and other reported cases of incontinentia pigmenti suggests that this diagnosis may adequately explain all the findings in our patient. Other authors have spoken of the possibility of viral infection during pregnancy as an etiologic factor, and Epstein [14] suggests searching for minor virus infections, such as herpes simplex. We now advise that such studies be done in all cases.

We have no satisfactory explanation for the apparent conversion of the tuberculin skin reaction from positive to negative. It does not seem likely that the relatively short course of antituberculous chemotherapy would accomplish this.

We have referred to the paucity of reports of this disease in pediatric sources. It is not mentioned in the major pediatric texts, and we believe that it deserves emphasis in the pediatric literature, because the patients are seen first by those caring for infants and children. Furthermore, because incontinentia pigmenti is in most instances a generalized disease, we suggest that, despite its rare occurrence, it be considered together with other generalized diseases exhibiting important skin manifestations.

Summary

A case of incontinentia pigmenti is presented exhibiting disturbances in the skin, teeth, eyes, nervous system, and growth pattern. The generalized aspects of this disease are emphasized. The concept of this disease as an infection, possibly with the herpes simplex virus, is discussed. This condition deserves more recognition in the pediatric literature as a generalized disease manifesting significant skin disturbances.

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Abstracts from Current Literature

Journal of Clinical Investigation, New York
Abstracted by Robert P. Igo, M.D.

36, No. 3 (March) 1957

° Plasma Protein Synthesis in the Human Fetus and Placenta. Joseph Dancis, Nancy Braverman, and John Lind.—p. 398.

Experimental Studies of the Mechanisms Producing Hypocalcemia in Hypernatremic States. Laurence Finberg.—p. 434.

Studies of Respiratory Physiology in the Newborn Infant: III. Measurements of Mechanics of Respiration. C. D. Cook, J. M. Sutherland, S. Segal, R. B. Cherry, J. Mead, M. B. McIlroy, and C. A. Smith.—p. 440.

A New Method for Measuring Sialic Acid Levels in Serum and Its Application to Rheumatic Fever. Eugene L. Hess, Alvin F. Coburn, Richard C. Bates, and Priscilla Murphy.—p. 449.

Stuart Clotting Defect: I. Segregation of an Hereditary Hemorrhagic State from the Heterogeneous Group Heretofore Called "Stable Factor" (SPCA, Proconvertin, Factor VII) Deficiency. Cecil Hougie, Emily M. Barrow, and John B. Graham.—p. 485.

Stuart Clotting Defect: II. Genetic Aspects of a 'New' Hemorrhagic State. John B. Graham, Emily M. Barrow, and Cecil Hougie.—p. 497.

36, No. 4 (April) 1957

The Renal Response in Man to Acute Experimental Respiratory Alkalosis and Acidosis. E. S. Barker, R. B. Singer, J. R. Elkinton, and J. K. Clark.—p. 515.

The Dynamics of Swallowing: I. Normal Pharyngeal Mechanisms. Michael Atkinson, Philip Kramer, Stanley M. Wyman, and Franz J. Ingelfinger.—p. 581.

The Dynamics of Swallowing: II. Neuromuscular Dysphagia of Pharynx. Philip Kramer, Michael Atkinson, Stanley M. Wyman, and Franz J. Ingelfinger.—p. 589.

A Heparin-Precipitable Fraction of Human Plasma: I. Isolation and Characterization of the Fraction. Richard T. Smith and Richard W. Von Korff.—p. 596.

° A Heparin-Precipitable Fraction of Human Plasma: II. Occurrence and Significance of the Fraction in Normal Individuals and in Various Disease States. Richard T. Smith.—p. 605.

36, No. 5 (May) 1957

Strontium^{85} and Calcium^{45} Metabolism in Man. Herta Spencer, Daniel Laszlo, and Milton Brothers.—p. 680.

The Distribution of Cr^{51} Labaled E. Coli Endotoxin in the Generalized Shwartzman Reaction. Richard T. Smith, Abraham I. Braude, and Francis J. Carey.—p. 695.

In Vivo Studies of the Role of Albumin in Endogenous and Heparin-Activated Lipemia-Clearing in Nephrotic Rats. Ray H. Rosenman and Meyer Friedman.—p. 700.

The Ultrafiltrable Calcium of Human Serum: I. Ultrafiltration Methods and Normal Values. T. Y. Toribara, A. Raymond Terepka, and Priscilla A. Dewey.—p. 738.

A and B Antigens in the Human Epidermis. D. Nelken, J. Gurevitch, and Z. Neuman.—p. 749.

36, No. 6 (June, Pt. 1) 1957

° The Response of Rheumatic and Nonrheumatic Children to Streptolysin O Concentrate. Robert W. Quinn.—p. 793.

Sodium Transport Across the Surface Membrane of Red Blood Cells in Hereditary Spherocytosis. John F. Bertles.—p. 816.

Proceedings of the 49th Annual Meeting of the American Society for Clinical Investigation, Held in Atlantic City, N. J., May 6, 1957.—p. 866.

36, No. 6 (June, Pt. 2) 1957

Metabolic Studies in Paralytic Acute Anterior Poliomyelitis: I. Alterations in Nitrogen and Creatine Metabolism. G. Donald Whedon and Ephraim Shorr.—p. 941.

Metabolic Studies in Paralytic Acute Anterior Poliomyelitis: II. Alterations in Calcium and Phosphorus Metabolism. G. Donald Whedon and Ephraim Shorr.—p. 966.

Metabolic Studies in Paralytic Acute Anterior Poliomyelitis: III. Metabolic and Circulatory Effects of the Slowly Oscillating Bed. G. Donald Whedon and Ephriam Shorr.—p. 982.

Metabolic Studies in Paralytic Acute Anterior Poliomyelitis: IV. Effects of Testosterone Propionate and Estradiol Benzoate on Calcium, Phosphorus, Nitrogen, Creatine, and Electrolyte Metabolism. G. Donald Whedon and Ephriam Shorr.—p. 995.

36, No. 7 (July) 1957

° Radioactive Iron Metabolism and Erythrocyte Survival Studies of the Mechanism of the Anemia Associated with Rheumatoid Arthritis. Emil J. Freireich, Joseph R. Ross, Theodore B. Bayles, Charles P. Emerson, and Stuart C. Finch.—p. 1043.

Acute Respiratory Illness in Volunteers Following Intramuscular Administration of Live Adenovirus. M. R. Hilleman; R. E. Hodges; M. S. Warfield, and S. A. Anderson.—p. 1072.

* Pulmonary Ventilation and Diffusion in the Human Newborn Infant. Mildred T. Stahlman.—p. 1081.
* Type-Specific Streptococcal Antibody. Floyd W. Denny, Jr., Wm. D. Perry, and Lewis W. Wannamaker.—p. 1092.
An Adaptation of the Nitrous Oxide Method to the Study of the Cerebral Circulation in Children: Normal Values for Cerebral Blood Flow and Cerebral Metabolic Rate in Childhood. Charles Kennedy and Louis Sokoloff.—p. 1130.
* Idiopathic Pulmonary Hemosiderosis: A Study of the Anemia and Iron Distribution Using Radioiron and Radiochromium. Leonard Apt, Myron Pollycove, and J. F. Ross.—p. 1150.

36, No. 8 (Aug.) 1957

The Distribution and Excretion of Phenobarbital. W. J. Wadell and Thomas C. Butler.—p. 1217.
Skeletal Muscle Analyses in Health and in Certain Metabolic Disorders: I. The Method of Analysis and the Values in Normal Muscle. B. A. Barnes, Esther B. Gordon, and Oliver Cope.—p. 1239.

36, No. 9 (Sept.) 1957

Acid Mucopolysaccharides in Normal Serum. Alfred Jay Bollet, Maria W. Seraydarian, and W. F. Simpson.—p. 1328.
* Thyroxine-Binding by Serum and Urine Proteins in Nephrosis Qualitative Aspects. Jacob Robbins, J. E. Rall, and Mary L. Peterman.—p. 1333.
* A Comparison of the Phenylalanine Content of the Hemoglobin of Normal and Phenylketonuric Individuals: Determination by Ion Exchange Chromatography. David W. Allen and W. A. Schroeder.—p. 1343.
Physiological Disposition of 4-C^{14}-Cortisol During Late Pregnancy. Claude J. Migeon, Jean Bertrand, and Patricia E. Wall.—p. 1350.
Influence of Cortisone and Prednisolone in Hyperbilirubinemia. Ricardo Katz, Hector Ducci, and Hernan Alessandri.—p. 1370.

36, No. 10 (Oct.) 1957

Radioactivity Over the Spleen and Liver Following the Transfusion of Chromium^{51} Labelled Erythrocytes in Hemolytic Anemia. Lee L. Schloesser, Donald R. Korst, Dallas V. Clatanoff, and Robert F. Schilling.—p. 1470.
Urinary Excretion of Acid Mucopolysaccharides by Patients with Rheumatoid Arthritis. Micola Di Ferrante.—p. 1516.

Abstracted by Robert L. Cranny, M.D.

36, No. 11 (Nov.) 1957

Plasma Lipid Interrelationships in Experimental Nephrosis. Ray H. Rosenman, Sanford O. Byers, and Meyer Friedman.—p. 1558.
* Biochemical Changes in Liver Associated with Kwashiorkor. Helen B. Burch, Guillermo Arroyave, Ruth Schwartz, Ana Maria Padilla, Motses Behar, Fernando Viteri, and Nevin S. Scrimshaw.—p. 1579.
Observations on the Minor Basic Hemoglobin Component in the Blood of Normal Individuals and Patients with Thalassemia. H. G. Kunkel, R. Ceppellini, U. Müller-Eberhard, and J. Wolf.—p. 1615.

36, No. 12 (Dec.) 1957

Studies of the Satiety Response in Mice. Guy Hollifield and William Parson.—p. 1638.
Capillary Observations in Patients with Hemorrhagic Fever and Other Infectious Illnesses. Sheldon Greisman.—p. 1688.

37, No. 1 (Jan.) 1958

The Ultrafiltrable Calcium of Human Serum: II. Variations in Disease States and Under Experimental Conditions. A. Raymond Terepka, T. Y. Toribara, and Priscilla A. Dewey.—p. 87.

37, No. 2 (Feb.) 1958

Calcium Metabolism in a Case of Gargoylism, Studied with the Aid of Radiocalcium. Felix Bronner, Clemens E. Benda, Robert S. Harris, and Joseph Kreplick.—p. 139.
* Thyroxine-Binding by Serum Protein in Pregnancy and in the Newborn. Jacob Robbins and James H. Nelson.—p. 148.
Studies on the Metabolism of Plasma Proteins in the Nephrotic Syndrome: II. The Lipoproteins. David Gitlin, David G. Cornwell, Doris Nakasato, J. L. Oncley, Walter L. Hughes Jr., and Charles A. Janeway.—p. 172.

37, No. 3 (March) 1958

* Studies on the Neonatal Development of the Glucuronide Conjugating System. Audrey K. Brown and Wolf W. Zuelzer.—p. 332.

37, No. 4 (April) 1958

Binding of Corticosteroids by Plasma Proteins: IV. The Electrophoretic Demonstration of Corticosteroid-Binding Globulin. W. H. Daughaday.—p. 519.
Further Studies of the "Pharmacology" of Placebo Administration. Louis Lasagna, Victor G. Laties, and J. Lawrence Dolan.—p. 533.

37, No. 5 (May) 1958

Observations on Carbon Dioxide Tension During Recovery from Metabolic Acidosis. Robert W. Winters, John A. Lowder, and Nelson K. Ordway.—p. 640.
* Albumin Metabolism in Children with Protein Malnutrition. David Gitlin, Joaquin Cravioto, Silvestre Frenk, Evangelina L. Montano, Rafael Ramos Galvan, Federico Gomez, and Charles A. Janeway.—p. 682.

An Analysis of Bicarbonate Reabsorption During Partial Inhibition of Carbonic Anhydrase. W. B. Schwartz, Adrian Falbriard, and Arnold S. Relman.—p. 744.

* Comparative Study of Glucose and Fructose Metabolism in Infants with Reference to Utilization and to the Accumulation of Glycolytic Intermediates. Robert Kaye, Margaret L. Williams, and Giulio Barbero.—p. 752.

Plasma Protein Synthesis in the Human Fetus and Placenta.—Electrophoretic studies of human fetal plasma have demonstrated a protein pattern in which all major fractions are present. The site of orgin of these plasma proteins is not known. The experiments presented were designed to investigate the synthesis of plasma proteins by the placenta and fetal liver.

It was concluded that liver slices from human fetuses of three to four months' gestation are capable of synthesizing plasma proteins exclusive of γ-globulin. Human placentas from pregnancies of three to four months gestation produced proteins with α- and β-globulin mobility. These were immunologically unrelated to plasma proteins. This was true also of term placentas. At neither stage of pregnancy does the placenta synthesize proteins that are electrophoretically identifiable as albumin or γ-globulin. It is concluded that under normal circumstances the placenta does not contribute significantly to the plasma proteins of the fetus. Beginning early in gestation, the fetal liver is capable of synthesizing all plasma proteins except γ-globulin.

A Heparin-Precipitable Fraction of Human Plasma: II. Occurrence and Significance of the Fraction in Normal Individuals and in Various Disease States.—The heparin-precipitable fraction (HPF) is a fraction of human plasma which precipitates at low temperatures in the presence of heparin. In apparently healthy persons the amount of HPF ranges from 0.07 to 0.27 gm. %, with the mean values varying slightly with age and sex.

Definitely elevated levels of the fraction appear to characterize inflammatory or necrotizing disorders in general rather than any specific disease process. The levels of the fraction in the plasma are roughly proportionate to the severity of the disease. Therefore, the clinical studies indicate that the occurrence of high levels of cold-insoluble, heparin-precipitable component in the plasma may be added to the long list of so-called "acute-phase" reactions occurring in the circulating blood of acutely ill patients. Elevations in this component roughly parallel the other acute-phase reactions, and it shows no unique advantages over other methods already in use.

The Response of Rheumatic and Nonrheumatic Children to Streptolysin O Concentrate. The authors administered streptolysin O concentrate to 20 children with rheumatic fever and 55 nonrheumatic children. The differences in the ASO titers prior to injection of the streptolysin O were not significant between the two groups. Three weeks after the injection of the streptolysin concentrate, the rheumatic children had significantly higher rises in ASO titer than the nonrheumatic children.

Six of the rheumatic and one of the nonrheumatic children had rather severe local and generalized reaction to the antigen. There was no evidence of initiation or reactivation of rheumatic fever. The authors state that the results indicate that, as a group, the children with rheumatic fever reacted more severely and had greater antistreptolysin O responses following the intramuscular injection of streptolysin O concentrate than nonrheumatic children.

Radioactive Iron Metabolism and Erythrocyte Survival Studies of the Mechanism of the Anemia Associated with Rheumatoid Arthritis.—After obtaining controls the authors administered plasma-bound radioactive iodine intravenously to 42 patients with rheumatoid arthritis to study iron metabolism. Thirteen patients with active rheumatoid arthritis were also studied for red cell survival. From the results of their studies of plasma iron utilization and red cell survival, the authors concluded that patients with rheumatoid arthritis show normal unimpaired use of plasma iron for erythrocyte production. However, rates of red cell destruction and red cell renewal are abnormally increased. The patient's erythrocytes are capable of normal survival in normal persons, indicating that the hemolytic mechanism is associated with the red cell environment rather than defective quality in the red cell. A defect in the release of iron from the reticuloendothelial system to the plasma, resulting in hypoferremia and an inadequate supply of iron for red cell production, contributes to the impaired capacity of the bone marrow for red cell production.

Pulmonary Ventilation and Diffusion in the Human Newborn Infant.—The purpose of this paper is to describe equipment and techniques designed to allow direct quantitative measurement of ventilation and diffusion in newborn infants. The steady-state carbon mon-

oxide method of Filley was chosen as a measure of diffusion property of the lungs. To keep dead space and resistance to expiration at a minimum in the measurement of carbon monoxide diffusion capacity in pulmonary ventilation in newborn, a double Donald-Christie box arrangement was chosen with 500 ml. counterbalance barometer interposed between the boxes. A tightly fitting mask was applied to the infant's face. Studies were carried out in 31 newborns and 12 older children and adults for comparison. Carbon monoxide uptake, alveolar ventilation, minute ventilation, oxygen uptake, and the diffusion capacity for carbon monoxide increase with the infant's body weight. Carbon monoxide uptake are shown to correlate well with alveolar ventilation in both infants and adults, and carbon monoxide uptake correlates well with oxygen uptake. The author states that although the method has inherent drawbacks as a measure of gaseous diffusion across the pulmonary alveolar-capillary membrane, it is thought that it can be applied to newborn infants in respiratory distress in an attempt to delineate types of pathological pulmonary physiology which occur in the newborn period.

Type-Specific Streptococcal Antibody.—Immunity to Group A streptococcal infections have been shown to be associated with the formation of antibody to the type-specific component of the Streptococcus, M-protein. Little is known concerning this antibody. Ninety-four patients were chosen for this study. Forty-nine patients with uncomplicated streptococcal infections were given no specific therapy. Twenty-two patients were treated with Aureomycin, and ten patients, with penicillin. The results of the test showed that the antibodies to the M-protein of the Streptococcus developed later than the antistreptolysin O antibodies and reached their peak much later than the peak for the antistreptolysin O antibody. The study also showed that patients receiving Aureomycin developed ASO titers as soon and to as great a degree as patients who received no therapy. Patients who received penicillin developed their ASO titers later, and 90% of the expected number developed rises in the ASO titers. Eighty-nine per cent of patients receiving no therapy developed type-specific M-protein antibodies. Seventy-four per cent of the patients treated with Aureomycin developed these antibodies, and none of the patients receiving penicillin developed these antibodies.

The data presented here show that Aureomycin in the doses employed is not as effective as penicillin in eradicating the Streptococcus or inhibiting the formation of the type-specific antibody.

Idiopathic Pulmonary Hemosiderosis: A Study of the Anemia and Iron Distribution Using Radioiron and Radiochromium.—Two patients with idiopathic pulmonary hemosiderosis were studied during periods of remission and exacerbation to determine the mechanism of the anemia. Erythropoiesis and movement of iron were studied with radioactive iron. Erythrocyte survival time was determined by labeling with radioactive sodium chromate and by the Ashby differential agglutination method.

During a period of remission, the plasma iron turnover in the metabolic pathway of radioiron was normal, and the survival time of the patients' erythrocytes in a healthy recipient and the life span of healthy donor's erythrocytes in the patient were normal.

During acute attack the path of radioiron was normal, but the movement was abnormally rapid. The plasma iron turnover was approximately triple the normal value. The survival time of the patients' chromated erythrocytes was normal when corrected for blood loss.

The studies indicate that during exacerbation excessive plasma iron turnover and iron kinetic pattern are characteristic of iron deficiency anemia and hemorrhage, but not of hemolysis. During remission there is no disturbance of erythropoiesis or erythrocytes survival. Iron which is lost from the circulating blood into the lung parenchyma is poorly reutilized despite a continuing iron-deficiency anemia.

Thyroxine-Binding by Serum and Urine Proteins in Nephrosis Qualitative Aspects.—Because patients with the nephrotic syndrome show hypercholesterolemia, lowered basal metabolic rate, and depressed serum protein, attention has been directed toward the thyroid gland in this disorder. Since the protein components of serum are extensively altered in nephrosis, a possible defect in this aspect of thyroid physiology has been suspected. The proteins from the sera of 11 patients were labeled by the addition of radiothyroxin in vitro, and, as in normal serum, thyroxin appeared to interact chiefly with two proteins, namely, albumin and an α-globulin (TBP). The TBP found in nephrotic sera appeared to be identical with the TBP of normal. At low concentrations of thyroxin, the thyroxin TBP complex accounted for most of the serum thyroxin. At high concentrations, TBP was saturated with thyroxin and the excess became associated with albumin.

The urine of one nephrotic patient was found to contain thyroxin in combination chiefly with TBP, and excess thyroxin added to this urine became associated with albumin. It was felt by the authors that the data presented in this report indicated that in the nephrotic state the serum proteins which interact with thyroxin are normal from a physical standpoint.

A Comparison of the Phenylalanine Content of the Hemoglobin of Normal and Phenylketonuric Individuals: Determination by Ion Exchange Chromatography.—Despite extensive clinical and biochemical research, the relationship of the metabolic error in phenylketonuria to the clinical symptomatology is unknown. One possible mode of phenylalanine toxicity would be through increased incorporation of phenylalanine in the body proteins and consequent interference with the proper functions of these molecules. The authors compared the phenylalanine contents in a pure protein, namely, hemoglobin, from patients with phenylketonuria and controls. The authors found by their work that if normal and phenylketonuric hemoglobin is of uniform phenylalanine content, they do not differ by as much as one residue of phenylalanine per molecule. It is possible that some of the molecules of the phenylketonuric hemoglobin have one or more extra residues of phenylalanine, but the accuracy of present techniques is unable to detect a difference in their average phenylalanine content of much less than one residue per molecule.

From this experimental evidence the phenylalanine toxicity in phenylketonuria is not associated with any abnormal accumulation of phenylalanine in hemoglobin.

Biochemical Changes in Liver Associated with Kwashiorkor.—Liver biopsy specimens, by needle aspiration, were obtained from 13 Guatemalan children with clinical kwashiorkor. Six of the cases were studied both before and after treatment. Four of the cases were classified as severe and the remainder, as moderately severe. All of the cases showed typical hair changes, skin lesions, edema, and apathy, and all had diarrhea. Analyses were made for xanthine, D-amino acid, and glycolic acid oxidases, DPNH-dehydrogenase, malic dehydrogenase, transaminase, riboflavin, total oxidized pyridine nucleotides, cholesterol, lipid, and protein in the liver, as well as the protein, cholinesterase, and amylase in serum and the riboflavin in red blood cells. Extensive experimental detail is presented.

A marked increase in liver protein and a marked decrease in liver total lipid was found to be associated with treatment. There were also striking increases relative to protein in xanthine oxidase and D-amino acid oxidase. During treatment, no significant changes relative to protein were found in liver riboflavin, glycolic acid oxidase, DPNH-dehydrogenase, malic dehydrogenase, transaminase, oxidized pyridine nucleotides, or cholesterol. The protein in serum was found to increase 70% during treatment and relative to protein, while cholinesterase increased 100% and amylase 30%. The erythrocyte riboflavin doubled.

No change in liver cholesterol was found in the cases studied, which, the authors note, is in contrast to a report by other workers in which high initial levels were found with a subsequent decrease following treatment. The finding of unchanged levels of hepatic malic dehydrogenase and transaminase activity after treatment agrees with the findings of other investigators. However, the absolute activity found by the authors for transaminase is 15 times as great as that reported by others. The authors feel that this discrepancy may be due to differences in methods.

Thyroxine-Binding by Serum Protein in Pregnancy and in the Newborn.—The authors studied thyroid function during pregnancy, immediately postpartum, in the newborn, and in nonpregnant adults. The three trimesters of pregnancy and the sixth postpartum week were studied separately. Five pregnant women were studied in serial fashion beginning as early as the second month of gestation. Total thyroxin, α-globulin binding sites for thyroxin, and free thyroxin were determined.

The data presented show that the thyroxin-binding α-globulin of serum (TBP), rose in the early weeks of pregnancy to a level which was approximately 2.5 that in normal adults, remained essentially unchanged throughout pregnancy, and returned to normal within six weeks post partum. The result of this rise in TBP was a significant lowering of free thyroxin in serum when compared to the levels in nonpregnant adults, despite a rise in serum PBI. The thyroxin-binding capacity of TBP in the newborn was found to be approximately one and one-half times that in normal adults but was lower than that in the mother. Since the PBI in the newborn was approximately equal to that in the mother, the concentration of free thyroxin was significantly higher. This level, however, was the same as that in nonpregnant adults.

The authors feel that the data favor the existence of an independently functioning fetal thyroid gland. The authors suggest that the difference between the concentration of TBP in maternal and fetal blood could be anticipated in view of differences in the concentration of other serum proteins.

Studies on the Neonatal Development of the Glucuronide Conjugating System.—The authors studied the enzyme systems involved in the glucuronic acid conjugation of bilirubin by fetal, newborn, and adult guinea pig livers. Direct bilirubin glucuronide synthesis was studied, using microsomal fractions as the source of glucuronial transferase and bilirubin orthoaminophenol and phenolphthalein as the glucuronial acceptors. The experimental procedures are given in detail. The authors found that bilirubin was conjugated to bilirubin-glucuronide when glucuronyl transferase was added to bilirubin in the presence of uridine-diphosphate-glucuronic acid. No bilirubin-glucuronide formations were demonstrated when the reaction mixture was incubated with microsomes from fetal or newborn guinea pig liver. Data were presented that indicate that glucuronide conjugation of phenolphthalein and orthoaminophenol also was poor or absent during fetal life and gradually increased during the postnatal period. Further data were presented to show the great increase in this enzyme system activity in adult guinea pig liver preparations as compared to fetal preparations. Mixture experiments utilizing adult and fetal soluble fractions and adult and fetal microsomes also demonstrated a great increase in UDPG dehydrogenase activity in adult guinea pig liver.

The authors feel that the data indicate an immaturity of the conjugating mechanism for bilirubin. This in turn could account for the accumulation of unconjugated bilirubin in the plasma of newborns. The authors further suggested that the enzyme system becomes comparable to that of the adult at about 15 to 20 days of age.

Albumin Metabolism in Children with Protein Malnutrition.—The authors investigated hypoalbuminemia associated with severe malnutrition by evaluating the synthesis, distribution, and degradation of intravenously administered I^{131} human serum albumin. Fourteen patients under the age of 7 years were studied. Serum albumin levels were determined for three weeks following the administration of the radioactive labeled material. This was followed by a second administration of intravenous I^{131} human serum albumin in eight of these patients. These eight patients were then followed for another three weeks with serial serum albumin levels.

The authors found that the half-life of albumin was not altered by diet or by the return of the plasma protein to normal. The initial hypoalbuminemia therefore was probably due to something other than an increase in protein catabolism. Since there was no loss of albumin in the urine, the authors conclude that the hypoalbuminemia was due primarily to a decrease in synthesis. It was pointed out that a decrease in caloric and protein intake in mice causes a decrease in protein synthesis which is partially compensated for by an increase in protein half-life. This was not the case in the patients studied.

In three of four patients with the nonedematous form of protein malnutrition, the initial serum albumin levels were less than many of those patients with the edematous form; therefore, the edema was not dependent solely on the serum concentration of albumin. The authors conclude that hypoalbuminemia in children with low protein intakes is probably due primarily to a decreased rate of albumin synthesis.

Comparative Study of Glucose and Fructose Metabolism in Infants with Reference to Utilization and to the Accumulation of Glycolytic Intermediates.—The study was designed to compare the utilization of glucose and fructose in infants at a level of intake which provides sufficient calories for caloric maintenance. The rates of administration were selected to determine whether fructose utilization equals the maximum generally considered to obtain for glucose (1 gm. per kilogram per hour) and to compare the utilization of the two sugars when administered at rates in excess of the infant's capacity for relatively complete glucose utilization (2 gm. per kilogram per hour). At the lower rate of infusion there was essentially complete retention of both sugars and no noteworthy differences were seen in the excretion of water, sodium, and potassium in relation to the sugar infused. When the infant's capacity was purposely exceeded, glucose administration was associated with significantly greater percentage of intake excreted in the urine (20%) than was fructose (9.9%). Sodium excretion was less (79% of intake) when associated with glucose than when associated with fructose infusion (127% of intake). No significant differences related to the sugar infused were found in the percentages of the potassium and water excreted in the urine. Statistically

significant alterations in pulse, respiratory rate, and liver size were noted during the infusion of fructose at the rate of 2 gm. per kilogram per hour. Further clinical details were presented.

Significantly greater rises in blood lactic and pyruvic acids in the subject receiving fructose than in those receiving glucose were noted. Accompanying the acid metabolite accumulation noted with fructose infusion were decreases in blood pH, in whole blood CO_2, and in plasma sodium. These decreases caused by fructose infusion were significantly greater than milder decreases associated with glucose infusion. No significant differences in potassium or chloride levels were demonstrated.

Extensive experimental data were presented.

The authors suggest that the advantage of fructose utilization over glucose, when administered at a rapid rate of infusion, may be offset by the induced metabolic acidosis and its attendant side-effects.

Journal of Pediatrics, St. Louis
Abstracted by Demetrius G. Traggis, M.D.

52, No. 3 (March) 1958

Suvren in Brain-Injured Children. N. Low and G. G. Myers.—p. 259.
The Diagnosis of Asthma in Infancy. W. P. Buffum.—p. 264.
Widened Roentgenographic Mediastinal Shadow (Thymus?) and Allergy in Childhood. J. Glaser, J. L. Aponte, and P. Barsky.—p. 267.
Group Sessions for Parents of Children with Epilepsy. G. J. Baus, L. Lavergne, and E. Russell.—p. 270.
The Trace Element Selenium and Its Influence on Dental Caries Susceptibility. D. M. Hadjimarkos and C. W. Bonhorst.—p. 274.
Testosterone-Induced Female Pseudohermaphrodism. A. P. Gold and A. F. Michael.—p. 279.
Cobalt-Induced Goiter with Cardiomegaly and Congestive Failure. Joseph A. Little and R. Sunico.—p. 284.
Tuberculous Meningitis in Children Treated with Isoniazidmethanesulfonate. H. Yoshida and Kozo Yanagawa.—p. 289.
Whirling as a Clinical Test in Childhood Schizophrenia. U. Kramer, R. Rabkin and R. L. Spitzer.—p. 295.
Cannula-Swab Technique for Obtaining Vaginal Secretions Painlessly in Young Girls. J. E. Thorner.—p. 304.
Current Management of Strabismus in Childhood. L. J. Lawson.—p. 307.
Generalized Osteomyelitis in a Newborn Infant. S. Z. Walsh and J. D. Craig.—p. 313.
Hamartoma of the Gall Bladder in a Child. R. C. Brown and R. J. Brown.—p. 319.
Acute Appendicitis in Early Childhood. F. Minervini and T. V. Santulli.—p. 324.
The Children's Medical Center, Boston. Clinical Pathological Conference.—p. 329.
* The Tonsil-Adenoidectomy Enigma. H. Bakwin.—p. 339.
Preparation of the Hemophilic Patient for Surgery: Use of Antihemophilic Globulin. R. J. Blattner.—p. 362.

52, No. 6 (June) 1958

Glycogen Storage Disease of the Heart. S. Friedman and R. Ash.—p. 635.
Further Studies on the Cardiovascular Status of Normal Newborn Infants: IV. Effect of Adrenaline, Acetylcholine, Ten per Cent Oxygen, and One Hundred per Cent Oxygen on the Electrocardiogram. C. Dupuis, B. Dupuis, F. H. Adams, J. Lind, and T. Peltonen.—p. 649.
True Hermaphroditism. W. Hughes, C. C. Erickson, W. Fleischmann, and J. N. Etteldorf.—p. 662.
Jacksonian Seizures in Infancy and Childhood. J. Holowach, D. L. Thurston, and J. O'Leary.—p. 670.
Vomiting in Infancy and Early Childhood: Treatment with Prochlorperazine. C. E. Hopkins and T. V. Geppert.—p. 687.
Transplacental Passage of the L. E. Factor. F. Mujer and R. N. Olsen.—p. 690.
Unexplained Death from Exchange Transfusion. W. C. Taylor, L. C. Grisdale, and A. G. Stewart.—p. 694.
Studies on Fetal Liver Functions in Homo- and Heterospecific Pregnancies with Special Reference to the Icterus Praecox of the Newborn. I. Halbrecht, P. Tiqua, M. Brzora, and M. Lahav.—p. 701.
Pseudomonas Aeruginosa Infections: Report of Two Patients with Meningitis. A. L. Hoyne, S. Metrick, and T. Sakuma.—p. 708.
Mental Deficiency, Spasticity, and Congenital Ichthyosis. J. K. Link and E. C. Roldan.—p. 712.
* A Reliable Paper-Strip Method for the Detection of Phenylketonuria. H. W. Baird.—p. 715.
The Mechanics of Establishing a Poison Control Center in a Community Hospital. S. C. Southard, W. B. Stewart, and W. F. Matthews.—p. 718.
The Children's Medical Center, Boston, Mass.—p. 723.
Recent Advances in Genetics in Relation to Pediatrics. F. C. Fraser.—p. 734.
Chelating Agents in the Treatment of Hepatolenticular Degeneration (Wilson's Disease). R. J. Blattner.—p. 758.

53, No. 1 (July) 1958

The Proper Use of Adrenal Hormonal Drugs in Pediatric Practice. A. M. Bongiovanni, W. J. Mellman, and W. R. Eberlein.—p. 3.
Pediatric Use of Antibacterial Agents. C. H. Kempe.—p. 19.
Iron as a Therapeutic Agent in Pediatric Practice. N. J. Smith.—p. 37.
Immunization Methods and Materials. R. Batson and A. Christie.—p. 51.
The Treatment of the Epileptic Child. J. G. Hughes and J. T. Jabbour.—p. 66.
The Management of Intestinal Parasitic Infections. R. C. Jung.—p. 89.
Treatment of Allergic Eczema Versus Seborrheic Infantile Eczema. L. V. Crawford.—p. 99.
The Children's Medical Center, Boston, Mass.—p. 107.
Obesity. R. S. Illingworth.—p. 117.
Environmental Penicillin and Penicillin-Resistant Staphylococcus Aureus. R. J. Blattner.—p. 131.

This issue of the *Journal of Pediatrics* contains very useful information which is of value to all pediatricians. There is too much to review in abstract form. It will be well worth the time of all physicians interested in the care of children to read this issue.

The Tonsil-Adenoidectomy Enigma.—Dr. Bakwin has brought out a lot of facts about an old problem, namely, T-and-A. He starts by citing the popularity of the operation. In general it is more prevalent with a better economic status. Rates may vary widely in nearby cities. It is hard to estimate the frequency of T-and-A. It was figured that in New York City in 1949 at least 45% of the children had had their tonsils removed. This has become even easier to do since health insurance plans seem to hasten the operation. Wolman's article on reasons for T-and-A was referred to, in which 13 reasons were given. In order of frequency they were recurrent or chronic tonsillitis and adenoiditis, prominent mouth breathing or snoring due to large size, recurrent or chronic tonsillitis, early hearing impairment, recurrent or chronic cervical adenitis, previous quinsy, recurrent or chronic asthma induced by respiratory infections, "tendency" toward rheumatic fever, recurrent or chronic sinusitis, recurrent or chronic lung infection, scarred or cryptic tonsils, large size alone, routine, and others. However, in actual practice the three major reasons were parental pressure, large tonsils and adenoids, and frequent URI. Probably the most enlightening study of the T-and-A problem was one in New York: 1,000 children 11 years of age were examined, and 61% were found to have had T-and-A's. The remaining 39% were reexamined by a group of school physicians, and 45% were selected for T-and-A. The rejected group was examined by another group of physicians, and 46% were selected for a T-and-A. The rejected group was examined by a third group of physicians, and 44% were selected for a T-and-A, leaving only 65 out of the 1,000, who were not reexamined because they had run out of doctors.

Another study referred to is by Epstein, who followed 540 children before and after T-and-A. He could find no correlation between size of tonsils, size of crypts, and amount of caseous material and the outcome of the operation. Nor was there correlation between histology, type and severity of preoperative symptoms, and the outcome of the operation. Size of adenoids did not correlate with the results of operation. Some constant mouth breathers had practically no obstruction of the nasopharynx (and surprisingly enough some of these were cured by operation).

Numerous comparison of tonsillectomized and nontonsillectomized children postoperatively reveal pharyngitis equally common, common colds more frequent in tonsillectomized; pneumonia and pleurisy commoner (though rare) in operated-on group; tonsillitis less frequent in operated-on group but bronchitis more frequent; according to one report, acute otitis media more frequent after adenoidectomy, and one observer (Mertz) found that, after age 40, persons with tonsils removed had a higher incidence of disabling respiratory illness. Comparison of hearing difficulty in children with and without operation is said by several writers to reveal no differences several years later. It was pointed out in several articles that allergic children have their tonsils removed not because of their allergy but because of the symptoms. Furthermore, a higher incidence of asthma is noted postoperatively. There was varied opinion concerning the postoperative results when cervical adenitis was the indication. It was also agreed that rheumatic fever was not per se an indication for T-and-A.

In discussing the hazards of T-and-A, a good description of the anesthetic dangers was given. It was pointed out that in a study conducted by several good hospitals (with excellent anesthesia departments) deaths from anesthesia for T-and-A amounted to 1:1,560. This is undoubtedly higher in less capable hands. In 1955 in the United States deaths from the operation numbered at least 220. The relationship between T-and-A and bulbar poliomyelitis was brought out. It was also pointed out in several articles that septicemia, although rare, still occurs in a sizable number of cases considering the number of operations performed. The psychological trauma is well recognized. A rough estimate of the total cost per year for T-and-A is $15,000,000.

After his review of the literature (given above) Dr. Bakwin concludes that in most cases T-and-A is unnecessary. He points out again the hazards enumerated above. He refers to the evidence citing little difference between tonsillectomized and nontonsillectomized persons five years later.

Comment: This article is, I believe, an important service to the medical profession. It was interesting and, in some respects, startling enough so that I reviewed all the journals

mentioned. One very interesting article was by Dr. John Fry in the *British Medical Journal*, Jan. 19, 1957, p. 124. He followed a large number of children for several years and found that they all had peaks of tonsillitis, cervical adenoiditis, otitis, etc. at about 6 to 8 years of age, but that after 10 years of age they were all doing well. This, he felt, is the natural course of infections in this age group. Le Riche and Stiver, in the *Canadian Medical Association Journal* (77:109, 1957), reported on a similar type of study and found much the same results. Actually there were less allergic symptoms in the non-T-and-A group. In reading over the article by Bordley and Hardy in *Acta otolaryngologica*, Supp. 120, 1955, I got the impression that these authors felt there was some benefit later on following irradiation of residual lymphoid tissue for hearing loss. The big problem with the article is that it left one at a loss to know who should have T-and-A's. Reading the article gives one the impression that there are no indications for it. As far as I can determine, otitis media and frequent tonsillitis, especially with cervical adenitis, are the chief indications for T-and-A.

A Reliable Paper-Strip Method for the Detection of Phenylketonuria.—The author reports on a simple test for the determination of phenylpyruvic acid in the urine. This test consists of saturating a specially prepared paper stick with urine and noting whether or not a color change occurs. The paper stick (now available commercially) is prepared by soaking it with ferric chloride and glacial acetic acid. If, when saturated with urine, it turns green or black, this indicates the presence of phenylpyruvic acid; a brown or white color is a negative test. The author tested various dilutions of phenylpyruvic acid in distilled water and in urine, specimens of urine from patients with phenylketonuria, and one case before and after a diet low in phenylalanine. The author found that urine containing 30 mg. % or more of phenylpyruvic acid gave a positive test, and in some instances urine containing 15-30 mg. % was positive. A false-positive test may occur in urines with excessive amounts of bilirubin. The author states that all children known to have phenylketonuria excrete more than 30 mg. % of phenylpyruvic acid in the urine after the age of 34 days. He recommends this test as a good screening procedure to be used on infants over 34 days of age. He reminds one that below 34 days of age one must rely on serum levels of phenylalanine to make the diagnosis.

Medical Journal of Australia, Sydney
Abstracted by George H. Lowrey, M.D.

1, No. 10 (March 8) 1958

The Management of Diabetes Mellitus in Children. S. E. Robertson.—p. 324.
The Plasma Ketone Test: Its Value in Diagnosis and Treatment. T. A. Heale.—p. 325.
Towards the Better Understanding of Physiological Acid-Base Balance. J. A. Owen.—p. 327.

1, No. 12 (March 22) 1958

* The Aetiology of Chronic Nephritis in Queensland. D. A. Henderson.—p. 377.

1, No. 13 (March 29) 1958

A Simple Method of Sweat Collection with Analysis of Electrolytes in Patients with Fibrocystic Disease of the Pancreas, and Their Families. Charlotte M. Anderson and Mavis Freeman.—p. 419.
The Treatment of Nephrosis. Donald G. Duffy.—p. 432.

1, No. 14 (April 5) 1958

Play Therapy. Marjorie Gilchrist.—p. 449.

1, No. 16 (April 19) 1958

Bornholm Disease in an Adelaide Suburban Area. L. Kaupmees and R. A. Pellew.—p. 517.
Epidemiological and Laboratory Investigations on Bornholm Disease in Adelaide, 1957. A. E. Duxbury and Peter Warner.—p. 518.

1, No. 17 (April 26) 1958

The Demonstration of the Carrier State in Christmas Disease. B. G. Firkin.—p. 557.
Pruritus Ani: A Prosaic Problem. Edward Wilson.—p. 558.
Indications for Removal of Tonsils and Adenoids. I. Lopert.—p. 561.
Tonsillectomy: Pathological Considerations. K. C. Porter.—p. 564.

1, No. 18 (May 3) 1958

A Case of Chronic Tracheal Obstruction Caused by a Foreign Body. Peter H. Sherwood.—p. 604.
Abstracts of papers presented at Paediatric Society of Victoria.—p. 615.

1, No. 19 (May 10) 1958

Pregnancy in the Diabetic. G. M. Parkin.—p. 622.

1, No. 20 (May 17) 1958

* The Ubiquitous Staphylococcus. V. D. Plueckhahn and Joan Banks.—p. 664.

1, No. 21 (May 24) 1958

* Some Aspects of Preventive Paediatrics. Donald Kerr Grant.—p. 693.
A Dual Role for Calomel in the Aetiology of Pink Disease. F. R. Barrett.—p. 704.

1, No. 22 (May 31) 1958

* Sugar and Dental Caries. C. Stanton Hicks.—p. 738.
Herpangina: Ulcerative Pharyngitis with the Isolation of Coxsackie Virus. Ian Jack and R. A. Chenoweth. —p. 741.

The Aetiology of Chronic Nephritis in Queensland.—This report is a summary statement of several papers concerning the subject of chronic nephritis. The incidence of the disease between 1876 and 1947 was much higher in Queensland than in any other area of Australia. All evidence points to the fact that a nephrotoxic agent was involved. Circumstantial evidence led to the implication of lead as the nephrotoxic agent. Among these are the high incidence of renal disease in persons known to have had lead poisoning in childhood, a significantly raised bone lead content in the group with chronic renal disease, a familial incidence of chronic renal disease, and a correlation between the bone lead values and the excessive mortality from renal disease in Queensland. The years in which the disease was prevalent coincide with the frequent use of lead paint in the area. Legislation in 1922 prevented the use of lead in paint, and this coincides with the decrease of mortality. The authors believe that this information has more than local interest, owing to the widespread incidence of plumbism.

The Ubiquitous Staphylococcus.—Twelve months' experience with staphylococcal infection in a maternity hospital is discussed. During this survey 194 babies were born. Staphylococcus aureus (coagulase-positive) was grown from 96% of babies aged more than 2 days. The areas most commonly yielding a positive culture were eyes, umbilicus, and skin, in that order. During this period over one-third of the babies born developed clinical evidence of Staphylococcus infection. Despite the usual measures to combat cross infection, the incidence of Staphylococcus remained high. The authors pointed out that "rooming-in" was of considerable value in cutting down the incidence of disease in the babies.
10346

Some Aspects of Preventive Paediatrics.—The author points out that in the larger cities of Australia, as in other countries, accidents are the largest cause of death among children, excluding the first few weeks of life. He also emphasizes that there is often over-treatment of minor or physiologic and anatomic variations in children. Among these, he mentions physiologic bowing of the legs, enuresis, and poor eating habits. He emphasizes that the clinican too often finds fault with the mother's methods of raising the child and does not give sufficient praise for things well done.

Sugar and Dental Caries.—The author points out that there are still many unknown features about the cause of dental caries. He believes that it is desirable to spread propaganda for the prevention of caries, if this is based on scientifically proven facts. The author reviews the available literature and points out several reports that indicate that if a good diet is eaten a substantial increase in the amount of sugar in that diet will not necessarily affect the child's liability to dental caries. This statement is based on observations made in England. Furthermore, the author says that evidence points to the fact that sugar intake is a real factor in caries activity only when it is consumed in a form which ensures that it sticks to and acts in a concentrated form on the surface of the tooth. "Sweetened drinks, on the other hand, can be consumed with impunity so far as sugar content is concerned."

Medizinische Klinik, Munich
Abstracted by Samuel Amberg, M.D.

53, No. 23 (June 6) 1958

* The Alleviation of Pain After Tonsillectomy in Children. E. M. L. Otto.—p. 1021.

53, No. 24 (June 13) 1958

The Drug Therapy of Speech Disorders, Particularly of Stuttering. E. Schönhärl.—p. 1055.

53, No. 25 (June 20) 1958

Actual Problems of Epidemiology and Pathogenesis of Poliomyelitis.—p. 1081.
The Relation of the Anatomic Lesions to the Clinic and Therapy of Poliomyelitis. M. Hertl.—p. 1085.

53, No. 26 (June 27) 1958

Heart and Circulation in Inflammatory Rheumatism. R. Günther.—p. 1124.

° Are Giant Cells in Nasal Smears an Aid for the Early Diagnosis of Measles? H. W. Ocklitz and R. Neuendorft.—p. 1130.

The Alleviation of Pain After Tonsillectomy in Children.—Microclysmas were given to children older than 5 years immediately after tonsilectomy by means of a "rectiole," containing 0.1 gm. phenobarbital, 0.15 gm. dimethylaminophenyl dimethylpyrazolon, and 0.15 gm. phenyldimethylpyrazolon in mucilaginous fluid. The relief lasted to the late evening or the next morning. If necessary one or two rectioles without the phenobarbital were used daily. An even better analgetic effect was obtained on addition of 5 mg. of codeine phosphate to the rectioles. The rectiole is made of plastic.

Are Giant Cells in Nasal Smears an Aid for the Early Diagnosis of Measles?—It had been claimed that the presence of giant cells in nasal smears allowed a diagnosis of measles before eruption of the exanthem. Eleven hundred four such smears from 40 children with measles and 130 controls did not confirm this claim. The method did not prove to be of value in the differential diagnosis.

Monatsschrift für Kinderheilkunde, Berlin
Abstracted by Hans Hartenstein, M.D.

106, No. 1 (Jan.) 1958

° Statistical Studies of Congenital Heart Disease. Wolfgang Callensee.—p. 1.
Capillary Resistance in Childhood, Especially When Using the Pressure Method. Susanne Loew and Johannes Oehme.—p. 8.
A New Method of Treating Interstitial Plasma-Cell Pneumonia of Premature Infants with Pentivalent Stibium and Aromatic Diamidines. G. Ivady and L. Paldy.—p. 10.
Dermographic Latency and Activity of Tuberculosis in Childhood. B. Leiber and H. Gotthardt.—p. 14.
° Results of Complement Fixation Reactions in Polimyelitis. E. von Liel and P. Schweier.—p. 18.
Isolated Colonic Tuberculosis and Chronic Regional Enteritis (Crohn) in Childhood. R. Ottenjann.—p. 22.
Review Article: Respiratory System. H. Hilber.—p. 29.

106, No. 2 (Feb.) 1958

° Evaluation of Success of the Dietary Treatment with Casein Hydrolysate Poor in Phenylalanine for Oligophrenia Phenylpyruvica. Rolf Gruettner, Ferdinand Mueller, and Hedwig Wa!lis.—p. 41-45.
Prognosis for Survival of Premature Infants (Seven and Eight Months Gestation) in Folklore and Ancient Theory: A Clinical Study. E. Pueschel.—p. 45-51.
Studies of Breast-Milk-Station Milk.—Gerhard Lindemann.—p. 52-55.
Clinical Studies of the Value of the Second Carbohydrate: 3. The Dispensability of the Second Carbohydrate with the Use of Evaporated Milk. L. Arp and T. Brehme.—p. 56-57.
Administration of Methyl-Naphthochinon and Icterus in Premature Infants. Joern Gleiss.—p. 58-62.
About the So-Called "Incomplete Right Bundle Branch Block" in the Electrocardiogram of Healthy Infants During the First One Hundred Days of Life. Walter Fuhrmann.—p. 62-69.
Use of X-Rays Above One Hundred KV in the Roentgen-Diagnosis of Childhood. Peter-Heinz Kocher.—p. 69-73.
Review Article: Genito-Urinary System. Johannes Oehme.—p. 73-79.

Statistical Studies of Congenital Heart Disease.—Between 1942 and 1955 average frequency of congenital heart anomalies at the University of Mainz Children's Hospital (Germany) was 0.79% of all patients admitted. Yearly increases were noted and attributed to the growing interest in such anomalies as well as to better diagnostic tools. In contrast to other authors, no correlation to higher maternal age was found.

Incidence of prematurity in patients with congenital heart disease was 23.5%; 4% of the affected patients were twins, as compared to a general incidence of 1.25% twin births. In 21.2% of patients other congenital malformations were detected, with Mongolism and situs inversis being most frequent.

There was a preponderance of boys in this series, which agreed with most other observations. Mortality was high, especially during the first few months of life.

Results of Complement Fixation Tests in Poliomyelitis.—Complement-fixation reactions with use of Hennessen's method were carried out on 53 patients with paralytic and 5

with nonparalytic poliomyelitis. All patients in the latter group had positive virus isolation studies. Complement-fixing antibodies could be demonstrated in 44 patients (76%). Virus isolation from stools was successful in 34 patients, of whom 23 had positive complement-fixation tests. Of these 23 patients, 13 showed the same type of virus isolated in stools as in the complement-fixation reaction.

It was thought that the complement-fixation test represented a valuable tool in the diagnosis of clinically doubtful cases of poliomyelitis. However, negative results of the complement-fixation test were frequent enough that such a negative test in a given doubtful case does not exclude the diagnosis of poliomyelitis.

Results with the complement-fixation test could be improved by obtaining a third blood specimen at a later date in case the first two samples showed negative results. Typing of polio virus by means of the complement-fixation test was not satisfactory in this study.

Evaluation of Success of the Dietary Treatment with Casein Hydrolysate Poor in Phenylalanine for Oligophrenia Phenylpyruvica.—The authors report on two boys, aged 2 years 3 months and 3 years 3 months, respectively, and one girl, aged 1 year 3 months, with oligophrenia phenylpyruvica, in whom determinations of the phenylpyruvic acid excreted in the urine were carried out and who were placed on a diet in which a casein hydrolysate to which tyrosine and tryptophan had been added was used as a protein source. The children received daily 2 to 2.5 gm. of this hydrolysate per kilogram of body weight. Fat was given in the form of coconut margarine and olive oil (daily amount of 3 gm. per kilogram of body weight) and carbohydrates, in the form of grape sugar and wheat starch (daily amount of 10 gm. per kilogram of body weight). Vitamin compound preparations and fruit juices also were given.

After six weeks of treatment with this diet, the excretion of phenylpyruvic acid in the urine was reduced from 187 mg. per 100 cc. to about 20 mg. per 100 cc. in the two younger children, i. e., the excretion of the phenylpyruvic acid was restored to normal. Control of the disturbance of metabolism thus was obtained in these two patients. In the 3-year-old boy, the excretion of the phenylpyruvic acid in the urine was considerably reduced but not restored to normal; the "lightening and nodding" cramps, however, subsided. Much emphasis has been put on the development of static and mental functions as an aid in the evaluation of the dietary therapy. A certain improvement in these functions was obtained in all three children, but the backwardness of the children remained so pronounced that severe idiocy was considered to be the right term for their condition. In the girl, a protein-deficiency syndrome occurred after three months of the dietary treatment, manifested by edema and reduction of the serum protein level to 3.2 gm. per 100 cc.; apparently mobilization of the autogenous protein resulted, which caused an increase in the pathological decomposition products of phenylaminopropionic acid (phenylalanine) and, therefore, an increased excretion of phenylpyruvic acid. The risk of such a complication has been stressed.

The authors agree with Armstrong and other workers that success of treatment with a diet poor in phenylaminopropionic acid may be expected only in children who are placed on this diet during the first months of life and in whom this treatment is continued throughout life. Irreparable damage of the substance of the brain must be taken into account when this therapy is instituted in the second or third year of life; normalization of the mental functions then has become impossible.

New England Journal of Medicine, Boston

Abstracted by Demetrius G. Traggis, M.D.

258, No. 12 (March 20) 1958

* Prevalence, Nature, and Identification of Leukocyte Antibodies. J. L. Tullis.—p. 569.
What Sets the Goals of Public Health? Sir G. Vickers.—p. 589.
Alloplasty in Surgery of the Eye. W. Stone Jr.—p. 596.
Effect of ACTH and Adrenocortical Steroids on External Pancreatic Secretion in Man. D. A. Dreiling, H. D. Janowitz, and H. Rolbin.—p. 603.

258, No. 15 (April 10) 1958

The Latex Agglutination and Inhibition Reactions: Clinical Experience in the Diagnosis of Rheumatoid Arthritis. A. P. Hall, A. D. Mednis, and T. B. Bayles.—p. 731.
Practical and Research Value of Hepatic Vein Catheterization. C. M. Leevy and M. L. Gliedman.—p. 738.
* Rubella Arthritis: Report of Cases Studied by Latex Tests. R. E. Johnson and A. P. Hall.—p. 743.
Current Concepts in Therapy.—p. 747.

258, No. 21 (May 22) 1958

The Shattuck Lecture: Studies on the Cause of Fever. W. B. Wood Jr.—p. 1023.
Hyperchloremic Acidosis in Chronic Pyelonephritis. W. Lathem.—p. 1031.
A Study of Illness in a Group of Cleveland Families: XV. Acquisition of Type-Specific Adenovirus Antibodies in the First Five Years of Life; Implications for the Use of Adenovirus Vaccine. W. S. Jordan Jr., G. F. Badger, and J. H. Dingle.—p. 1041.
Contributions of Open Cardiotomy to the Correction of Congenital and Acquired Cardiac Disease (to be concluded). C. W. Lillehei.—p. 1044.

258, No. 22 (May 29) 1958

Buccally Administered Streptokinase: Clinical and Experimental Observations. I. Innerfield, H. Shub, and L. J. Boyd.—p. 1069.
Acute Benign Pericarditis: Report of Two Cases Associated with Group A and Group B Coxsackie Viruses. E. R. Movitt, E. H. Lennette, J. F. Mangum, M. Berk, and M. S. Bowman.—p. 1082.
Contributions of Open Cardiotomy of the Correction of Congenital and Acquired Cardiac Disease (concluded). C. W. Lillehei.—p. 1090.

258, No. 23 (June 5) 1958

Pulmonary Alveolar Proteinosis. S. H. Rosen, B. Castleman, and A. A. Liebow.—p. 1123.
X-ray Findings in a Child with Candida Esophagitis. H. J. Kaufmann.—p. 1143.
Iron-Deficiency Anemia Resulting from Fetomaternal Transfusion. J. J. McGovern, R. Driscoll, C. H. DuToit, M. Grove-Rasmussen, and R. F. Bedell.—p. 1149.
Salt Intake and Salt Need. L. K. Dahl.—p. 1152.

258, No. 24 (June 12) 1958

Meprobamate Habituation: A Controlled Clinical Study. T. M. Haizlip and J. A. Ewing.—p. 1181.
Hepatodiaphragmatic Interposition of Jejunum, Ileum, Cecum and Ascending Colon with Intestinal Obstruction: Report of a Case Corrected Surgically. D. W. Aiken.—p. 1192.
Pulmonary Schistosomiasis. H. F. Rodriguez and E. Rivera.—p. 1196.
The Seventeenth Veterans Administration Armed Forces Conference on the Chemotherapy of Tuberculosis. E. Rothstein.—p. 1199.
Salt Intake and Salt Need (concluded). L. K. Dahl.—p. 1205.
Liver Damage Concurrent with Iproniazid Administration. L. Zetzel and H. Kaplan.—p. 1209.
Current Concepts in Therapy.—p. 1213.

258, No. 25 (June 19) 1958

Mass Prophylaxis of Epidemic Streptococcal Infections with Benzathine Penicillin G: I. Experience at a Naval Training Center During the Winter of 1955-56. A. J. Shreier, V. E. Hockett, and J. R. Seal.—p. 1231.
Effects of Varying Infusion Time in Maintenance Fluid Therapy. O. Neyzi, M. Bailey, and N. B. Talbot.—p. 1239.
° Fulminating, Rapidly Fatal Shigellosis in Children. D. Hoefnagel.—p. 1256.

259, No. 4 (July 24) 1958

Enhancement of Tetracycline Blood Levels. C. M. Kunin, W. F. Jones, and M. Finland.—p. 147.
Hematologic Complications Arising During Ristocetin Therapy: Relation Between Dose and Toxicity. E. J. Gangarosa, N. S. Lenderman, P. J. Rosch, and E. G. Herndon.—p. 156.
Studies in Ammonia Metabolism: IV. Biochemical Changes in Brain Tissue of Dogs During Ammonia-Induced Coma. G. M. Clark and B. Eiseman.—p. 178.
Antihistamine Therapy for Gingival Hyperplasia Due to Diphenylhydantoin. J. Holowach, D. L. Thurston, and J. E. Gilster.—p. 180.
° Brief Recording: Clinical Paralytic Poliomyelitis Due to Coxsackie Virus Group A, Type 7. E. R. Ranzenhofer, F. C. Dizon, M. M. Lipton, and A. J. Steigman.—p. 182.

258, No. 26 (June 26) 1958

Mass Prophylaxis of Epidemic Streptococcal Infections with Benzathine Penicillin G: II. Experience at a Naval Training Center During the Winter of 1956-57 R. B. McFarland, V. G. Colvin, and J. R. Seal.—p. 1277.
Transfusion Reactions from Contaminated Blood: Their Recognition and Treatment. A. I. Braude.—p. 1289.
Polycythemia Vera (concluded). G. M. Pike.—p. 1297.
A Peroxidase-Staining Procedure for the Identification of Polymorphonuclear Leukocytes and Leukocyte Casts in the Urinary Sediment. M. Kaye.—p. 1301.
° Early Jaundice in the Newborn: Aids to Detection. F. H. Allen Jr.—p. 1302.

Prevalence, Nature, and Identification of Leukocyte Antibodies.—The author refers to reports in the literature concerning the identification and importance of leukocyte antibodies. He then presents a test for leukocyte antibodies. The reader is referred to the article for the details, but the essentials are as follows: 1. White cells from a Type O, Rh-negative donor are mixed with complement from a Type O, Rh-negative donor and the serum to be tested. 2. The cells are counted at 0, 30, and 60 minutes, and the percentage decrease is noted. 3. Ameboid activity is noted at 0 and 60 minutes, and the percentage decrease is

noted. 4. The percentage decrease in number times the percentage decrease in ameboid forms is called the lysis index, and with normal sera it is less than 25. Seventy sera were found strongly positive (lysis index, average 630), and 20, weakly positive, 6 of which were from patients with multiple transfusions.

Forty-five percent of leukopenic states tested were strongly positive. With bone marrow aplasia the test was usually negative. Of 18 cases with primary idiopathic agranulocytosis (splenic neutropenia), 11 sera were positive before therapy (cortisone and/or splenectomy). The positive test suggested that cortisone and/or splenectomy would be beneficial. Of 37 cases of hypersplenism, 31 showed positive tests (idiopathic, 13; Hodgkin's disease and other lymphoma of spleen, 8; Felty's syndrome, 3, and miscellaneous, 7). It was noted that hypersplenism with thrombopenia only (no leukopenia) gave negative tests. In cases of drug toxicity 10 of 18 sera from patients who did not have aplasia of the bone marrow were positive. No patients with drug toxicity and aplasia of bone marrow gave positive tests. With steroid treatment of the positive tests "dramatic response was shown." Four positive sera were found in the aleukemic leukemia group (three in acute histiocytic leukemia). Of 20 weakly positive sera, most of the patients had had transfusions.

Of 102 cases of blood dyscrasia, 11 sera were positive (5 in acute and chronic hemolytic anemia and the remaining 6 in diseases associated with abnormal circulating globulins, granulmatous liver disease, plasma-cell leukemia, and lymph node hyperplasia).

Several identification procedures were carried out. Exposure of a positive sera to an excess of antigen (leukocytes) was found to remove the antileukocyte activity. Electrophoresis revealed a major peak in β-globulin and a lesser one in α-globulin. In tests using the properdin system it was found that properdin was anticomplementary. When the serum was fractionated according to Pillemer's method, the antileukocytic factor was thermolabile. In its native state it is thermostable.

Comment: This article is interesting, but the procedure appears to depend on meticulous care in counting technique.

Rubella Arthritis: Report of Cases Studied by Latex Tests.—The authors report on 10 cases of arthritis associated with rubella. According to them there are numerous reports of the condition in the foreign literature but few in the United States literature. It is a disease chiefly of young adults, predominantly girls. Joint pains, with swelling, appear with or shortly after the onset of rash and last up to a few weeks. Temperature may be elevated but may be normal with severe polyarthritis. Hands, wrists, and ankles are usually involved. Generalized muscle and joint pains without swelling are common.

Of the authors' 10 cases, all were girls and the swelling started in the fingers. Fingers, wrists, knees, ankles, elbows, and feet were involved, in frequency of order stated. The authors did latex-fixation studies on the patients. Nine of ten were positive for inhibition; two of ten, positive for euglobulin, and none, for agglutination. Of seven cases of uncomplicated rubella, two were positive for the inhibition and none for the euglobulin and serum agglutination. The latex-inhibition test is the most sensitive, and false-positives tests may result in about 5.2% of patients with nonrheumatoid arthritis and in one-third of persons with no history of joint disease but with family histories of rheumatoid arthritis (from an article by Hall, Mednis, and Bayles, *New England Journal of Medicine*, same issue, p. 731).

The authors speculate as to the possibility that a virus may be at least one cause of rheumatoid arthritis.

Fulminating, Rapidly Fatal Shigellosis in Children.—The author reports nine fatal cases of shigellosis. Death occurred from 8 to 46 hours after onset. Ages ranged from 1 3/12 to 8 years, mostly under 3 years. Three of the nine had temperatures of 107 F or a little over. None of the nine had convulsions. Symptoms were sudden onset, with nausea, lethargy and temperature, and occasional vomiting. Bloody foul-smelling diarrheal stools were passed by some before admission. None appeared to be severely dehydrated or to have cardiac failure or respiratory distress. Blood cultures and spinal fluid were negative. Urine was negative. WBC was elevated, with a preponderance of neutrophils. Death appeared associated with peripheral vascular collapse in most. Autopsy examinations revealed changes in the bowel, especially the lower intestinal tract. No findings to indicate the cause of death.

The author refers to other reports in the literature of fulminating fatal cases. Because several of his cases appeared in siblings he recommended that prophylactic sulfadiazine be administered to others in the family.

Brief Recording: Clinical Paralytic Poliomyelitis Due to Coxsackie Virus Group A, Type 7.—The authors report a case of a 3-year-old boy who developed fever, stiff neck, vomiting, and "stiff-legged walking." Physical examination showed nuchal rigidity. No paralysis was present. Spinal fluid revealed 245 cells (68% polymorphonuclears). Protein 32 mg., sugar 56 mg/100 ml., and chloride 121 mEq/L. WBC 13,000, with 58% polymorphonuclears. Day after admission right lower facial paralysis and generalized weakness developed. Four days after admission the temperature was normal (five days of fever), but the right leg showed paralysis. Twelve days after admission the facial paralysis was improving, but the right leg was very weak, as were hip muscles on the left. In a year's follow-up these improved so that only residual paralysis of the right foot was left.

A virus, Coxsackie Group A, Type 7, was isolated from stool injected into suckling mice, but there was no growth in tissue culture. Sera revealed no neutralizing antibody to all three seratypes of poliomyelitis virus. With use of an in vivo test of the serum in suckling mice it was found that the protective titer of the admission serum against Coxsackie Group A, Type 7, was 1:6, whereas the serum drawn two weeks later showed a protective titer of 1:630.

In discussing the case the authors referred to a Russian report of paralysis associated with the same virus. This strain was found to cause paralysis in monkeys by the Russians and others. The authors also refer to unpublished work noting that nonpoliomyelitis enteric viruses may occasionally cause paralysis in humans.

Comment: This case report is valuable in calling attention to the fact that supposedly relatively benign viruses may cause serious illness and furthermore that whenever an epidemic that has similarities to polio occur, it should not be labeled as polio until proven. There is a marked tendency to label any disease in which are found a stiff neck and cells in the spinal fluid that occurs in the summertime as polio, leading to much apprehension in the community and often casting doubt as to the usefulness of the polio vaccine.

Early Jaundice in the Newborn: Aids to Detection.—The author refers to the importtance of detecting jaundice in the newborn. He mentions the various causes and points out that about the only one that can be suspected before birth is erythroblastosis due to the Rh factor. The deleterious effect of indirect bilirubin on the central nervous system is reiterated. At the Children's Hospital several steps are taken to make the nursing and staff personnel aware of the problem and to make the detecting of jaundice easier. Essentially they include a special tag on the bassinet, the correct type and amount of illumination, and the use of a special lucite stick to be used in blanching the skin of the cheeks and forehead to make the detection of jaundice easier.

Comment: The author has brought out some very practical points in his discussion of the correct illumination. His emphasis on making people aware of the necessity and methods for detecting early jaundice is well taken.

Pediatria, Moscow
Abstracted by Moses Grossman, M.D.

36, No. 3 (March) 1958

Chronic Tonsillitis in Preschool Children. A. V. Dolgopolova.—p. 11-16.
Hypertensive Disease in Children and Adolescents. V. S. Gvantseladze, N. S. Chochua, T. E. Karrvelishvili.—p. 17.
Study of Nailbed Capillaries in Juvenile Hypertension. L. T. Antonova, and V. A. Volodina.—p. 21.
° Phonocardiography of the Normal Heart in Children. M. K. Oskolkova.—p. 25.
The Wolff-Parkinson-White Syndrome in Early Childhood. R. S. Novoselova.—p. 33.
Atrioventricular Dissociation with Interference in Childhood. B. N. Semenov and V. N. Makarov.—p. 39.
Growth and Development of the Two- and Three-Year-Old Child: Normal Standards. R. B. Kogan.—p. 49.
Nonspecific Antihyaluronidase in Children with Active Rheumatic Fever. M. S. Ignatova.—p. 66.

Phonocardiography of the Normal Heart in Children.—One hundred healthy children between the ages of 8 and 14 years were studied phonocardiographically. The following results were noted.

Splitting of the first sound occurred in 9% of the cases, splitting of the second sound, in 19%. The third heart sound was present in 55%. The fourth (atrial) sound was heard in 25% of cases. Duration of sound was as follows: First sound, 0.1-0.16 second (average 0.1 second); second sound, .05-0.1 second (average 0.08 second); third sound, 0.03-0.05 second (average 0.03 second).

The interval between the beginning of the second and beginning of the third sound is 0.12-0.2 second (average 0.14 second).

Seventeen per cent of these children registered slight systolic murmurs which were felt to be innocent ones.

Pediatrics, Springfield, Ill.
Abstracted by William W. Cleveland, M.D.

21, No. 2 (Feb.) 1958

Respiratory Illness and an ECHO Virus. H. G. Cramblett et al.—p. 167.
Effect of Low-Phenylalanine Diet on Phenylketonuria. D. Y.-Y. Hsia et al.—p. 178.
Accidental Ingestion of Estrogens. R. Hertz.—p. 203.
Aortic Stenosis. P. A. Ongley et al.—p. 207.
Malignant Melanoma. J. R. Derrick et al.—p. 222.
Hepatic Fibrosis and Concentration of Bilirubin in Atresia of the Biliary Tract. R. A. Christy et al.—p. 226.
Penicillin in the Serum Following Administration of Benzathine Penicillin G. R. I. Lade et al.—p. 233.
Local Tolerance to Benzathine Penicillin G. S. Krugman et al.—p. 243.
Congenital Defects in Biosynthesis of Thyroid Hormone. H. D. Mosier et al.—p. 248.
Congenital Abnormalities of Intestinal Innervation. J. D. Boggs et al.—p. 261.
Methacholine in the Management of Paroxysmal Tachycardia. G. S. Husson.—p. 267.
Proteins in the Cerebrospinal Fluid in the Newborn. R. G. Arnhold et al.—p. 279.
* Infant Feeding Practices. H. F. Meyer.—p. 288.
Chondroectodermal Dysplasia. H. L. Smith et al.—p. 298.
Epileptic Equivalents. C. H. Snyder.—p. 308.
Public Health: The Practical Value of the Tuberculin Test. American Academy of Pediatrics.—p. 319.
Tranquilizing Drugs. C. Bradley.—p. 325.
Clinical Conference: A Complication of Nephrosis.—p. 337.

Infant Feeding Practices in Hospital Maternity Nurseries: A Survey of 1,904 Hospitals Involving 2,225,000 Newborn Infants.—A survey by questionnaire regarding feeding practices in 1,904 hospitals and representing 2,244,667 infants during 1956 is reported. The report is particularly concerned with the instances in which one milk mixture is used for most infants in a nursery, constituting a so-called house formula. Of the hospitals reporting, 82.1% reported the use of a standard house formula. The milk product upon which this formula is based was found to be evaporated milk in 65.8%, premodified prepared formulas in 30.9%, pasteurized milk in 3.1%, and pasteurized skimmed milk in 0.2%. In this group of nurseries, it was found that 21% of the infants were breast-fed, 16% were breast- and bottle-fed, while 63% were completely bottle-fed. Also of interest was the report that, of the hospitals reporting, 817 received a free supply of milk products from the manufacturer, while 921 received carbohydrate additives free. The limitations of this type of study are discussed, as is the significance of the findings as regards infant feeding.

Comment: This article stimulated an editorial comment by Dr. Charles D. May, which appears in this same issue. Particular emphasis was placed on the practice of hospitals receiving products free from the manufacturer, and physicians are urged to abolish this practice. He concludes, "after all, who pays for the free supply?"

Zeitschrift für Kinderheilkunde, Berlin
Abstracted by Gunnar B. Stickler, M.D.

81, No. 1 (May) 1958

Lupus Erythematosus in Childhood. H. Eggers.—p. 5.
* Contribution to the Clinical Picture of the Infantile Progressive Spinal Muscular Atrophy (Werdnig-Hoffmann Atrophy). P. C. Schmid.—p. 13.
Influence of Cystine Feeding on Tissue Amino Acids and α-Amino-N in Kidney Blood. U. Stave.—p. 26.
Ergometric Testing as a Criterion of Cardiac and Physical Efficiency in Children and Adolescents. H. Mellerowicz and D. Lerche.—p. 36.
Unilateral Pulmonary Agenesis with Esophageal Atresia. B. S. Schultze-Jena and W. Kosenow.—p. 50.
Complications During Exchange Transfusions in Newborn and Premature Infants. U. Keuth.—p. 68.
Half-Life and Total Clearance of Inulin and PAH in Infancy. U. Keuth and A. Neumann.—p. 80.
Differential Diagnosis of the Congenital Factor VII-Deficiency. A. Burmeister.—p. 88.
Subendocardial Fibroelastosis with Eosinophilia (Löffler) in a 7-Year-Old Child: First Description in the Pediatric Age Group. D. Knorr and K. J. Scheppe.—p. 102.
* Indications for and Duration of Oral INH Therapy Alone in Tuberculous Meningitis. H. Versé.—p. 113.

Contribution to the Clinical Picture of Infantile Progressive Spinal Muscular Atrophy (Werdnig-Hoffmann Atrophy).—The author describes the disease in three siblings in whom the diagnosis was substantiated by electromyography. Two of these children had the

disease in a relatively mild form. He emphasizes the variability of the clinical findings and course. The author also points out that it appears to be impossible to differentiate this disease from amyotonia congenita.

Indication and Duration of Oral INH Therapy Alone in Tuberculous Meningitis.—This is not a carefully controlled study and the number of cases is too small. However, of 19 children with tuberculous meningitis in its first two stages (not when associated with motor paralysis) 16 were "clinically cured," that is, were without relapse for one to three years, by treatment with INH given by mouth in a daily dose of 10 mg. per kilogram of body weight for 12 months. Three of these nineteen patients died.

Abstracts from the Soviet Literature

A selection of abstracts drawn from the current issue of Abstracts of Soviet Medicine, *a quarterly journal published by the Excerpta Medica Foundation (New York Academy of Medicine Building, 2 E. 103d St., New York 29), with the aid of a grant from the U. S. Public Health Service*

Newborn Period, Prematurity, Embryopathy

Paresis of the larynx caused by birth trauma (Parez gortani na pochve rodovoi travmy.) TATOCHENKO Z. A. *Vopr. Okhr. Mater. i Dets.* 1956, 1/6 (79-81)

A female infant of 3500 g. weight and 50 cm. length was born in a state of asphyxia (a double twisting of the umbilical cord round the neck). The birth took place at term assisted by low forceps. The baby stayed in the maternity home 20 days; weight gain was poor and attacks of cyanosis, screaming and lowering of muscle tone were observed. On the third day after discharge the condition of the baby deteriorated sharply; breathing was disturbed. A direct laryngoscopy helped in diagnosis of a paresis of the left half of the larynx caused by a trauma of the CNS. Treatment proved unavailing; death ensued. The anatomical diagnosis was: atelectases and foci of catarrhal pneumonia in both lungs, a moderate stenosis of the pulmonary artey with hypertrophy of the wall of the right ventricle; petechial haemorrhages in the arachnoid; haemorrhages in the cerebral substance of the right parietal region; congestion and dystrophy of the internal organs. The multiplicity of haemorrhages in the cerebral substance made it impossible to localize with precision the site of the lesion.

The method of early autoplacental blood injections as a prophylactic and therapeutic measure in the treatment of intracranial haemorrhages in the newborn (Metod rannikh inektsii autoplatsentarnoi krovi kak profilaktika i lechenie vnutricherepnykh krovoizliyanii u novorozhdennykh.) KANTOROVICH L. N., BOGOMAZOVA L. M. *Zdravookhr. Belor.* 1956, 12 (23-26)

Timely injections of autoplacental blood are indicated in all cases of deviation from the normal in labour accompanied by traumatization of the foetus or stasis contributing to the rupture of vessels. Injections of autoplacental blood were administered early to 542 newborns (1st group); a control group contained the same number of cases. In the group of newborns who had injections of autoplacental blood, the over-all mortality in traumatized newborns was 5.9% as against 12.5% in the control group, a definite clinical picture of haemorrhage was noted in 6.4% of the first group and in 16.2% of the controls; death from intracranial haemorrhage occurred in 2.4% of the 1st group and in 7% of the controls. The injection of autoplacental blood at birth lessens the total mortality by 2.5 times, the mortality from intracranial haemorrhage nearly 3 times, and morbidity 2.5 times; in addition, these injections improve the general state of the baby traumatized during birth.

Gastrointestinal System

Metabolism of adrenaline in children with dysentery (K voprosu ob obmene adrenalina u detei, bolnykh dizenteriei.) OZERETSKOVSKAYA N. E. and CHERNIKOVA A. P. *Vopr. Pediat.* 1956, 4 (56-62)

In children with acute infections changes have been noted in the activity of the higher nervous system and in the reactivity of the vegetative nervous system; there is predominance of the function of the sympathetic nervous system in the first days of the illness and of the parasympathetic subsequently. The high tonus of the sympathetic nervous system in the acute period is possibly a defensive reaction of the body (protective inhibition in the cerebral cortex and increase in activity of the vegetative centres in the sub-cortical region).

Factors which inhibit the functioning of the sympathetic nervous system in the acute stage of disease aggravate the course of the disease by depressing the body's physiological defense mechanism. In children with dysentery the concentration of adrenaline in the blood varies during the whole course of the disease. At the beginning, free adrenaline is found in the blood and dehydroadrenaline is absent; during clinical improvement and especially during recovery the concentration of free adrenaline diminishes and that of dehydroadrenaline grows. If complications or secondary toxicosis occur, the concentration of dehydroadrenaline decreases. The administration of large quantities of ascorbic acid is of great importance in setting free adrenaline from its combination with protein and in converting dehydroadrenaline into free adrenaline.

Liver, Spleen, Pancreas, Peritoneum

Infectious hepatitis in children (Epidemicheskii gepatit (bolezn Botkina) u detei.) FLEKSER S. YA. and NAUMOVA M. A. *Vopr. Pediat.* 1956, 4 (38-44)

The clinical course of infectious hepatitis was studied in 171 children in the No. 2 (Rusakov) children's hospital in Moscow during 1954-1955. Cases were admitted to hospital late, only 28 children being admitted in the pre-icteric stage. In cases seen early at the polyclinic the practitioners made wrong diagnoses (acute gastroenteritis, tonsillitis, food poisoning, etc.). In the pre-icteric stage (lasting 7-10 days) a brief rise of temperature was usually seen, falling on the 2nd-3rd day or pyrexia of an irregular character for 3-5 days, followed by malaise and anorexia. In half the cases there was abdominal pain and vomiting. In the majority of patients there was leucopenia but in 4 cases leucocytosis. Bradycardia was not always marked. The icteric stage began on the average after a week, lasting 15-30 or more rarely 40-70 days. The general condition improved, and toxic signs began to disappear. The blood bilirubin was 2.56-10.24 mg./100 ml. or higher. Since there is no specific treatment for infectious hepatitis the authors recommend a rational diet starting with two 'unloading days' (sour milk, sugar, fruit). For the milk-fermentation, strains of acidophilus bacilli were chosen which eliminate the putrefactive microflora of the intestine. This milk was then added to the 'liver menu' together with a sufficiency of easily assimilated first class proteins, carbohydrates and vitamins but only small quantities of fats. In addition, general therapy was used (glucose, insulin, campolon, plasma, etc.).

Nutrition, Deficiency Diseases

The effect of diet with an increased egg-content on the physical development and conditioned reflex activity of healthy children of pre-school age (Vliyanie ratsiona s povyshennym soderzhaniem yaits na fizicheskoe razvitie i uslovno-reflektornuyu deyatelnost' zdorovykh detei doshkolnogo vozrasta.) ARSHAVSKAYA E. I., VEDRASHKO V. F. and RATMANOVA O. N. Institute of Nutrition, USSR Academy of Medical Sciences, Moscow *Vopr. Pitan.* 1956, 15/4 (13-18) Illus. 3 Ref. 8

Thirty children aged 6-7 years were under observation. The overall content of protein, fats and carbohydrates in both of the 2 test diets was identical. When an egg was substituted for part of the meat in diet nr. 2, an insignificant change in the amino-acid content of the protein was observed. A more considerable change was observed in the lecithin content. In diet nr. 1 lecithin constituted 2,300 mg., while diet nr. 2 had 4,508 mg. The addition of yolk to the diet almost doubled the lecithin content. It was this which constituted a factor determining the change in the child's conditioned reflex behaviour. In children who received diet nr. 1 the whole time, a certain preponderance of the stimulatory process over the inhibitory was noted, as well as a worsening of the mobility of nervous processes when they were examined for a second time in the spring. In children on diet nr. 2, however, all these phenomena were more pronounced. Without discounting the possibility that the season had its effect on the children's higher nervous activity, it must be admitted that, on increasing the quantity of yolk in the diet, the increase in the stimulatory processes was more strongly pronounced than in children on diet nr. 1, in which there was only half an egg. For this reason, children of pre-school age should not be given two eggs daily over a prolonged period of time.

Krymskii—Moscow

Hypervitaminosis D in breast-fed infants (O gipervitaminoze D u grudnykh detei.) BLASOV V. A. and STOLYAROVA V. K. *Vopr. Okhr. Mater. i Dets.* 1957, 2 (11-15)

Observations were made on 10 children aged 4-12 months. To prevent rickets these children were given an alcoholic solution of vit. D_2 in doses several times in excess of the usual

permissible doses. The highest total dose in one instance was 13 million units. This treatment was continued for 1-4 months. The degree of intoxication was not directly related to the amount of vitamin taken. Several children received daily, in addition to the alcoholic solution of vitamin D_2, also vitamin-enriched fish fat, and two children also a course of ultraviolet irradiation. The symptoms of intoxication appeared 1-3 months after the start of vitamin treatment. They developed gradually. Some central nervous system symptoms were noted: somnolence and apathy, or restlessness, insomnia, ready perspiration, decrease or complete loss of appetite, increased thirstiness, frequent vomiting, and constipation; a dry, peeling skin; dilatation of the heart, dull heart tones, a transient systolic murmur, tachycardia, elevated blood pressure; enlargement of liver, and in some cases also of spleen. In the urine the presence of albumen, leucocytes and occasional erythrocytes and casts was noted. An increased concentration of calcium (14-18-23 mg./100 ml.) was observed also. The ECG showed changes in the ventricular myocardium in all the children; roentgenologically there was a widening of calcification zones in the epiphyses of the shin and forearm bones, and in some cases a thickening of the cortical layer of the long bones. The authors strongly recommend adherence to the established dosage in prescribing vitamin D_2, and to use fatty solution of it in small doses, and not to prescribe vitaminized fish fat and ultraviolet irradiation simultaneously with vitamin D_2.

Acute Infectious Diseases

Antitoxic immunity in scarlet fever patients treated with penicillin (Sostoyanie antitoksicheskogo immuniteta u bolnykh skarlatinoi, lechennykh penitsillinom.) NISEVICH N. I. and ISMAILOVA M. KH. *Vopr. Okhr. Mater. i Dets.* 1956, 4 (28-32)

Following at attack of scarlet fever, children elaborate a high antitoxic immunity to the toxin of the haemolytic streptococcus. When treated with penicillin under conditions which prevent reinfection (cubicles or filling the ward with patients at one admission) the children are cleared of haemolytic streptococci within the first 48 hours. In such children a much lower level of antitoxic immunity is produced than in those not treated with penicillin. Antitoxic immunity (negative Dick test to one skin test dose) is present by the 30th day in almost all children, but high immunity (negative to 4 skin test doses) is met with only half as often in children treated with penicillin as in the untreated. Very high immunity occurs only in occasional children. The weak immunological reactivity with penicillin treatment is due to the rapid clearing of haemolytic streptococci from the body and not to a depression of general immunological reactivity of the body. In order to protect children from serious complications of scarlet fever penicillin treatment must be used extensively even if this leads to some increase in repeated infections.

The significance of penicillin and other factors in the causation of repeated attacks of scarlet fever (K voprosu o znachenii penitsillina i drugikh faktorov v vozniknovenii povtornoi skarlatiny.) BEREZINA L. A. and MAUERMAN O. E. *Pediatriia* 1956, 4 (17-22)

540 children who have had scarlet fever twice and 16 who have had it three times have been traced. A second attack of the disease was more common in those children who had incurred the first attack before the age of two. In such cases the physical development has been retarded and nutrition poor. Half of all the children suffered from chronic diseases such as tonsillitis, otitis, tb, dysentery, etc. The clinical course of the second attack of scarlet fever was mild in 81% of cases. The interval between the first and second attacks was 1-5 years. Short intervals were three times more common in those children who were put on penicillin from the first day of the disease than in the case of those who did not receive it until in the later stages. The incidence of the second attacks was 4 times more common in those treated with penicillin than in those to whom it was not administered.

Chronic Infectious Diseases

The value of fundus examinations in children suffering from miliary tuberculosis and tuberculous meningitis (Znachenie issledovaniya glaznogo dna u detei, bolnykh miliarnym tuberkulezom i tuberkuleznym meningitom.) BEREZHANSKA T. S. and PILMAN N. I. *Pediat. Akush. i Ginek.* 1957, 2 (29-31)

106 children aged 3 months to 16 years, suffering from miliary tb and tb meningitis, were observed. In 85.5% of them there were pathological changes in the fundus oculi. Ophthalmological examinations revealed dissemination of the process. Tubercles in the choroid were observed more frequently in younger children. If treatment procedures are not adequate, the healing of the tubercles proceeds slowly, sometimes taking up to 2 years to complete.

Usually the resolution process of the tubercles and the production of new disseminations take place concurrently. In some cases the appearance of tubercles in the choroid is the only sign of an exacerbation of the tuberculous process.

Rheumatic Diseases

Functional status of the higher regions of the CNS at various stages of rheumatism in children (Funktsionalnoe sostoyanie vysshikh otdelov tsentralnoi nervnoi sistemy pri raznikh etapakh razvitiya revmaticheskogo protsessa u detei.) KHODAK K. M. *Trud. II Sezda Vrac.-pediat. USSR* 1956 (261-264)

Children with articular rheumatism in the acute stage showed extensive inhibition at the cortical and at the subcortical level. Following an attack, there was a tendency to normalization of cortical processes. In the cardiac form of rheumatism the acute phase was characterized by a heightening of subcortical excitation and by inhibition in the cortex. In the quiescent phase of articular and cardiac forms of rheumatism uniform changes in functionality of the CNS were observed. In chorea, at the time of abatement of the hyperkinesis, there was a considerable cortical inhibition, with marked excitation at subcortical levels. The functional deviations in the CNS remained unchanged during the quiescent period. In order to arrest the progress of the disease and to reduce mortality, it is necessary to augment the preventive and therapeutic measures taken after and between acute phases of the disease.

Surgery, Roentgenology

Surgical treatment of congenital pyloric stenosis in breast-fed infants (Operativnoe lechenie vrozhdennogo pilorostenoza u grudnykh detei.) TERNOVSKII S. D. and ROZANOVA N. A. *Pediatriia* 1957, 4 (73-77)

Ninety cases seen between 1946 and 1955 are discussed. Clinical signs are described, and peristalsis of the 'hour-glass' stomach quoted as a constant and pathognomonic sign. The severity of the condition may be estimated objectively by the daily loss of weight as a percentage of the weight of the infant at birth. Diagnostic errors made by the referring practitioners are specified. Infants with congenital pyloric stenosis were sent to hospital as cases of dyspepsia, dysentery, insussusception, pneumonia, otitis; they were often referred very late (out of the 90 not less than 50 infants were admitted to hospital more than 3 weeks after the onset of the disease). The Fredet-Ramstedt operation is considered to be the only efficient surgical method, and should be performed on all infants with pyloric stenosis without regard to the degree of wasting. Fifty-seven infants weighed at the time of operation less than when they were born, and many of them had some concomitant complaint (e. g. pneumonia, otitis); nevertheless the operation was in most cases successful after 2 to 4 days preoperative preparation (intravenous drips of fluids, plasma, blood). Fatal outcome (3.3%) took place in cases of gross wasting, and when the infant was referred too late. Forty-eight children were kept under follow-up observation for a period of 5 months to 6 years. One of them died from pneumonia, all the others progressed satisfactorily without showing any clinical or radiological signs of gastro-intestinal disorder.

The diagnosis and surgical treatment of patent ductus arteriosus (O diagnostike khirurgicheskom lechenii otkrytogo arterial'nogo protoka.) PETROVSKII B. V. and KESHISHEVA A. A. *Khirurgiia* 1957, 1 (6-15)

Operation was performed on 26 out of 46 patients with patent ductus arteriosus. In order to establish the diagnosis, in addition to the usual clinical examinations there were also performed venous angiocardiography and cardiac catheterization with measurement and recording of intracardiac pressure. Angiography with serial photographs was performed on 32 patients. The most accurate diagnostic data are given by aortography which should only be performed when strongly indicated (in 7 cases). Simultaneous appearance of the contrast medium in the aorta and pulmonary artery is pathognomonic. In 26 patients on whom operation was carried out the ductus was doubly ligated in 25 cases, with no occurrence of recanalization. In one patient the duct was divided. All patients recovered after operation. The late results (under observation from 6 months to 3 years) showed that in all cases of isolated patent ductus arteriosus surgical intervention was followed by recovery of the patients. The optimal age for surgical operation is 3-4 years. In the presence of marked clinical signs of the lesion, surgical treatment at an earlier age is indicated.

Social Medicine, Prevention, Organization

Prospective tasks in pediatrics following resolutions of the All Union Convention of Public Health Workers (Blizhaishie zadachi v oblasti pediatrii v svyazi s resheniem Vsesoyuznogo aktiva rabotnikov zdravookhraneniya.) SOKOLOVA-PONOMAREVA O. D. *Pediatriia* 1957, 2 (3-8)

In order to diminish the mortality and morbidity among children it is necessary to improve the organization of prophylactic measures in towns and villages. Division into small medical units for child care is considered effective. Combining nurseries with kindergartens is recommended as ensuring uninterrupted observation of the child's progress. In maternity homes attention should be paid to the prevention of obstetrical injuries. Breast feeding and regularity in feeding should be widely propagated among the population. A child should receive a regular supply of dry milk products and a wide variety of fruit and vegetable preserves; canteens for children and hot breakfasts should be organized in schools. Urgently needed are convalescent wards in children's hospitals for children recovering from dysentery. New prophylactic and therapeutic methods should be sought in the campaign against infectious diseases.

News and Comment

ANNOUNCEMENTS

Celebrating Golden Jubilee of Children's Hospital in Hawaii.—The Honolulu Pediatric Society is sponsoring the Golden Jubilee Celebration of Kauikeolani Children's Hospital from April 19 to 22, 1959, in Honolulu. Guest participants for the scientific program will be Drs. J. Roswall Gallagher, Chief Adolescent Unit, Children's Medical Center, Boston, and C. Tague Chisholm, Assistant Clinical Professor of Surgery, University of Minnesota. The topics will be centered on problems of adolescent and pediatric surgery. Reservations may be made with the Golden Jubilee Committee, Children's Hospital, Honolulu, Hawaii, or directly through the A. M. A. Academy of Pediatrics c/o E. H. Christopherson.

Princess Kaiulani Hotel Meeting House will be the center for the scientific sessions.

Intermountain Pediatric Society Meeting.—The Intermountain Pediatric Society announces its annual meeting, to be held at Sun Valley, Idaho, June 26, 27, and 28. The program is to be presented by the following speakers: Robert Alway, M.D., M. E. Lahey, M.D., Ralph Platou, M.D., and Josef Warkany, M.D. Sessions will be held daily from 8 a. m. until noon. Round table daily from noon until 1:30 p. m. The meeting is open to all physicians. For further information please write to Intermountain Pediatric Society, 2000 South 9th East, Salt Lake City, Utah.

Residency in Pediatric Oncology.—Applications for the Residency from Jan. 1, 1960, to Dec. 31, 1960, in Pediatric Oncology at Memorial Center for Cancer and Allied Diseases in New York will be received up to May 1, 1959. This residency is approved for partial credit by the American Board of Pediatrics and is limited to the neoplastic diseases of children. Salary: $3,600 annually plus maintenance. For application forms and information write to Director, Pediatric Service, Memorial Center for Cancer and Allied Diseases, 444 E. 68th St., New York 21.

The 27th annual series of the Benjamin Knox Rachford Lectureships will take place in Cincinnati, on Tuesday and Wednesday evenings, April 21 and 22, 1959, at 8:30 p. m. Dr. Lorimer Dods, Professor of Child Health at the University of Sydney, Australia, will deliver these lectures in the auditorium of the Children's Hospital Clinic and Research Building. On April 21 Dr. Dods will lecture on "Pediatrics in the Antipodes," and on April 22 his subject will be "Some Pediatric Philosophy."

Postgraduate Seminar for Pediatricians.—The Eighth Annual Post-Graduate Seminar for Pediatricians will be conducted at St. Christopher's Hospital for Children, of the Pediatric Department of Temple University School of Medicine, Philadelphia, May 20-23, 1959. Half-day sessions will be devoted to various phases of pediatric medicine.

For information regarding enrollment, registration fee, etc., write to Dr. John B. Bartram, St. Christopher's Hospital for Children, 2600 N. Lawrence St., Philadelphia 3.

Course in Pediatric Oncology.—The Pediatric Department of Memorial Center for Cancer and Allied Diseases announces that the annual comprehensive three-day course in Pediatric Oncology for pediatricians, general practitioners, and health officers, will be held April 29, 30, and May 1, 1959.

Current developments and established methods in diagnosis, differential diagnosis, and management of benign and malignant tumors, Hodgkin's disease, leukemia, and reticuloendotheliosis in childhood are included.

Content of Course: Ward rounds, seminars, demonstrations, examinations of children in Pediatric, Surgical, Chemotherapy, Radiotherapy Clinics.

Faculty: Twenty members of the attending staffs of Memorial Hospital and Sloan-Kettering Institute for Cancer Research.

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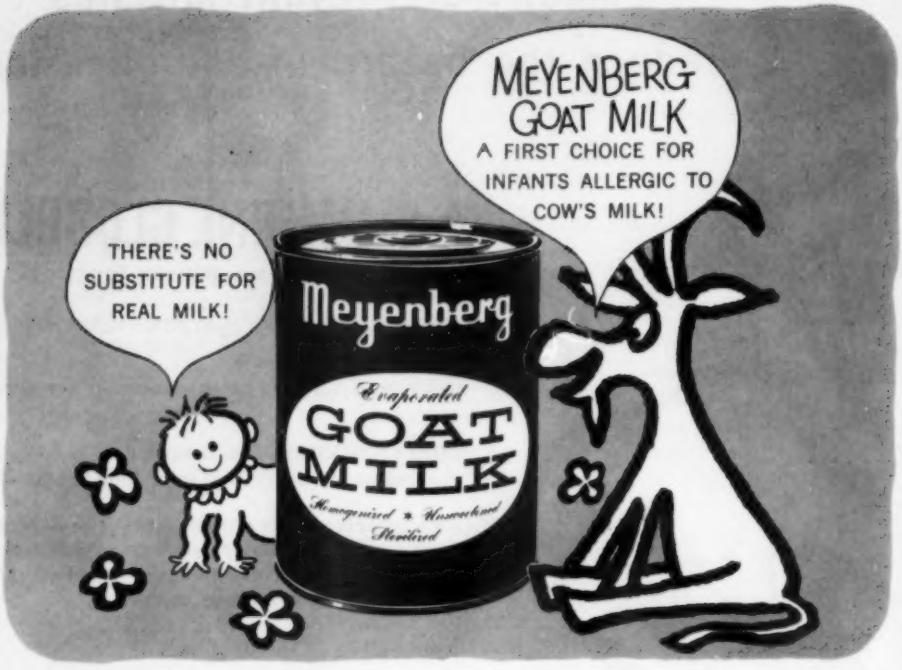

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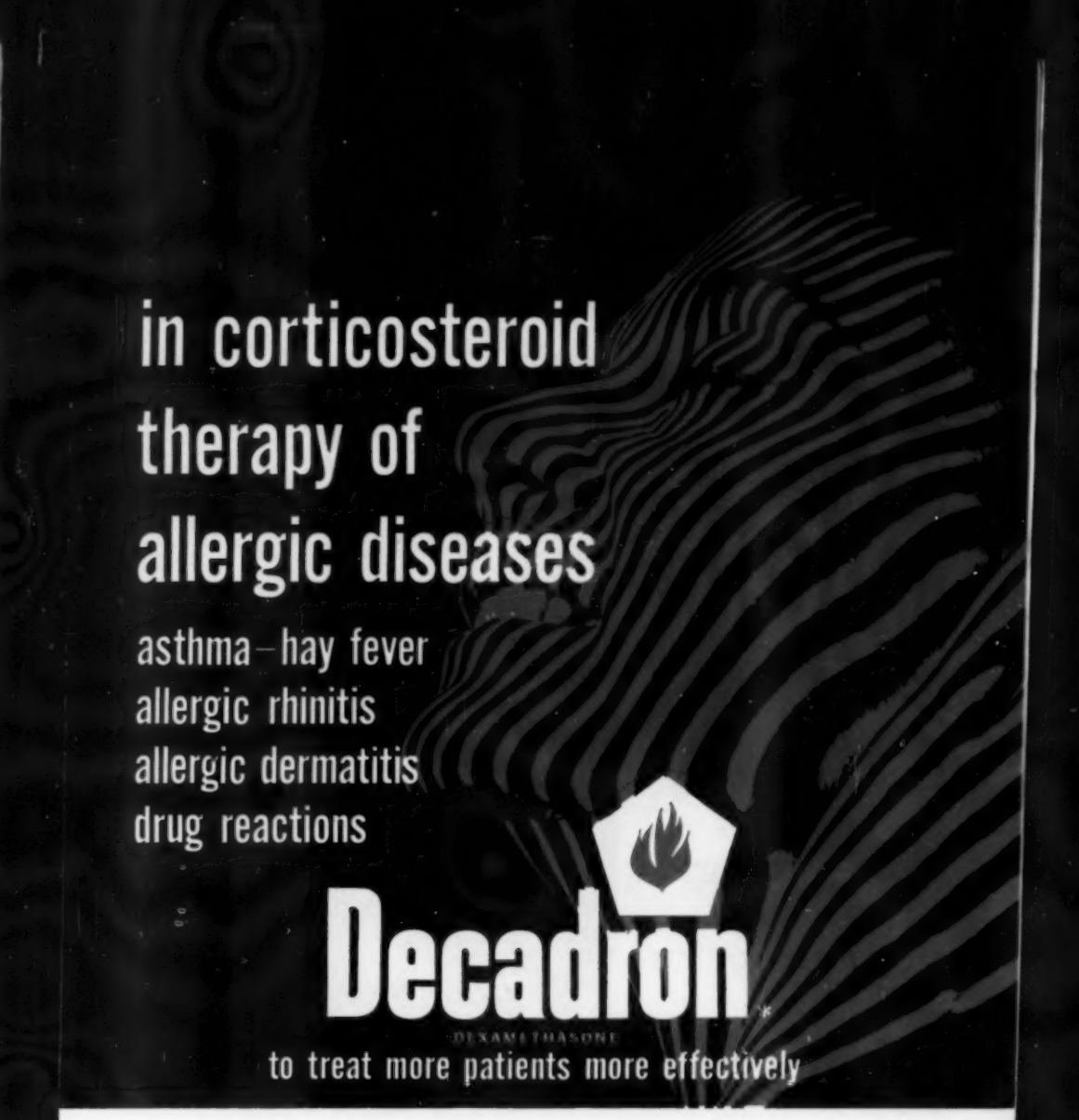
*Fries, J. H., Milk Allergy-Diagnostic Aspects and the Role of Milk Substitutes, J.A.M.A., Nov. 23, 1957
**Ibid.



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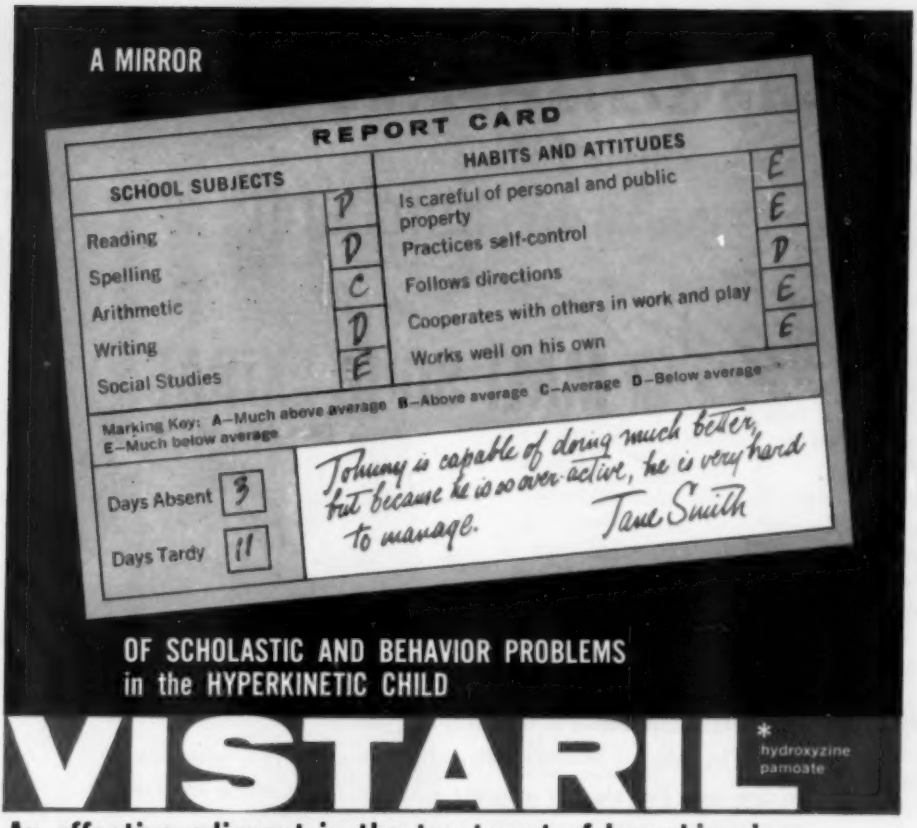

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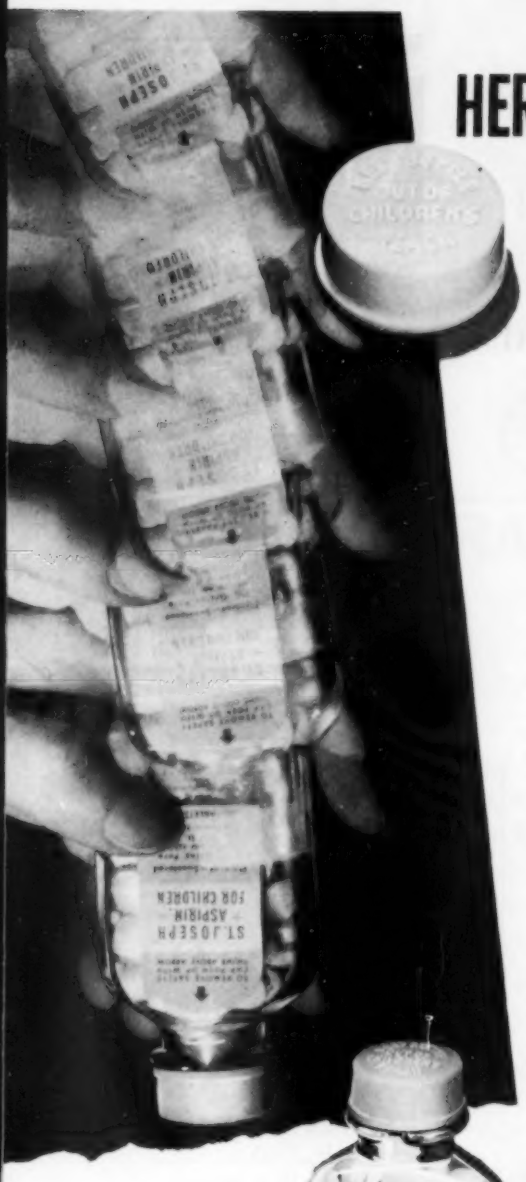

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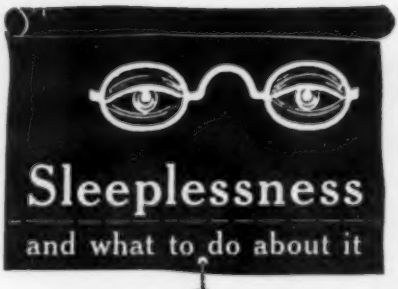

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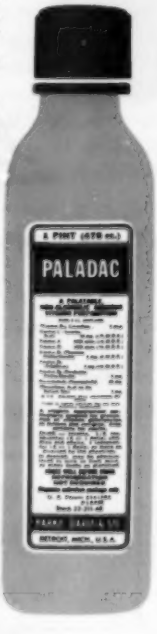
PALADAC®

liquid multivitamin formula

is orange in color, aroma, and flavor because orange has specific meaning for children. From the time they first taste orange juice and later learn to enjoy orange-flavored drinks, orange ice, and orange candy, orange is a universal favorite—appealing, palatable, highly acceptable.

PALADAC is a 9-vitamin formula that is even-flowing, readily miscible, if desired, with milk, fruit juice, or other foods, and is stable, requiring no refrigeration.



Each 4-cc. teaspoonful of PALADAC supplies:

Vitamin A	5,000 units
Vitamin B_1 (thiamine hydrochloride)	3 mg.
Vitamin B_2 (riboflavin)	3 mg.
Nicotinamide (niacinamide)	20 mg.
Vitamin B_6 (pyridoxine hydrochloride)	1 mg.
Pantothenic acid (as the sodium salt)	5 mg.
Vitamin B_{12} (crystalline)	5 mcg.
Vitamin C (ascorbic acid)	50 mg.
Vitamin D	1,000 units

Available in 4-ounce and 16-ounce bottles.

PARKE, DAVIS & COMPANY
DETROIT 32, MICHIGAN 88199

little

How to win friends ...

The Aspirin that Tastes So Good.

The Flavor Remains Stable down to the last tablet.

25¢ Bottle of 48 tablets.

We will be pleased to send samples on request.

THE BAYER COMPANY DIVISION

of Sterling Drug Inc.

1450 Broadway, New York 18, N. Y.

The Value of Oatmeal Protein

in the Dietotherapy of Undernutrition

Following periods of catabolic stress—when rehabilitation by diet is especially important—many patients cannot tolerate certain high-nutrition foods, and frequently show an aversion to foods with high-fat content.

Under such circumstances the physician is faced with the problem of selecting nourishing foods which are easily digested and well tolerated. Each day's menu must be built from a variety of high-protein, high-nutrient foods which appeal even to jaded appetites.

Oatmeal is an outstanding source of natural nutrients well suited for debilitated patients. It ranks highest among whole-grain cereals in content of protein, thiamine, and phosphorus.

The oatmeal serving with milk provides high-quality protein, unimpaired in processing or preparation. It supplies the essential amino acids in proper proportions for biosynthesis, repair, and replenishment of tissue. Oatmeal also provides significant amounts of several B vitamins and minerals important to physiologic rehabilitation.

The smooth, semi-solid texture of oatmeal, the ease of its digestibility, its inviting warmth, and its delicious nut-like taste combine to make it an appealing dish to patients of all ages.

Quaker Oats and Mother's Oats, the two brands of oatmeal offered by The Quaker Oats Company, are identical. Both brands are available in the Quick (cooks in one minute) and the Old-Fashioned varieties which are of equal nutrient value.

IN PEDIATRIC NUTRITION, oatmeal, with its easy digestibility and assimilation, and its wealth of essential nutrients, merits recommendation among the first solid foods as soon as spoon-feeding can be instituted.

The Quaker Oats Company
CHICAGO

"Ah, to Breathe Again!"
Open spaces are no help in breathing, when nasal passages are shut tight from pollen or with head colds.
Neo-Synephrine®
HYDROCHLORIDE
Pediatric Nasal Spray
0.25% • 20 cc.
contains Zephiran® chloride 1:5000, antibacterial wetting agent and preservative for greater efficiency
In the handy plastic squeeze bottle
Prompt and Prolonged Decongestion
• Usually no compensatory vasodilation
• No sting
• Virtually no systemic reactions
PEDIATRIC
NASAL SPRAY
NEO-SYNEPHRINE® HCl
Brand of phenylephrine HCl
20 cc. SOLUTION 1/4%
with Zephiran® Cl 0.02%
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Neo-Synephrine (brand of phenylephrine) and Zephiran (brand of benzalkonium, as chloride, refined), trademarks reg. U. S. Pat. Off.